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Investigation of Dental Caries Using Quantitative Light-induced Fluorescence (QLF).

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy

By

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Abbreviations

CCD	Charge-coupled device.
ΔF	% of fluorescence loss compared to sound enamel.
ΔQ	Integrated value of ΔF over lesion area in mm^2.%.
ΔR	Red/ green fluorescence ratio.
EDJ	Enamel dentinal junction.
GF	Green fluorescence.
ICDAS	International Caries Detection and Assessment System.
LAC	Linear Attenuation Coefficient.
μCT	X-ray Microcomputed Tomography.
μm	Micrometer.
mm^2	Millimetre squared.
PC	Personal Computer.
QLF	Quantative Light-induced Fluorescence.
RF	Red fluorescence.
SPSS	Statistical Package for the Social Sciences.
TMR	Transverse Microradiography.
USB	Universal Serial Bus.
WS	White spot.

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Dedication

To my wonderful husband, Salah and my lovely children, Faris, Hala and Faisal who made PhD dream true.

Declaration

This thesis is the result of my own work. The material contained in the thesis has not been presented, nor is currently being presented, either wholly or in part for other degree or qualification.

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ABSTRACT

The prevalence of dental caries overall has declined in many European countries but this has not occurred equally for all tooth surfaces and the percentage of the people who are dentate has subsequently increased. This has resulted in an amplification of the number of natural teeth at risk of developing dental caries. This makes site specific diagnosis of dental caries even more important, especially on the occlusal surfaces which are commonly affected. An increased proportion of total caries encumbrance is found in fissures. This perhaps results from superficial remineralisation potential of low concentrations of fluoride over extended periods.

Several methods of caries diagnosis have been used but most rely on the dentist's subjective interpretation of clinical outcomes. Restorative repair of consequences of dental caries is costly in terms of time, resources, and oral health. Prevention of demineralisation and the endorsement of remineralisation of early caries is the main focus of contemporary dentistry. However; these goals can only be achieved if caries is detected at an early stage.

QLF parameters mean little to dentists and as with any new technology; QLF presents a challenge for the specialist. Many *in vitro* studies and some *in vivo* studies had been conducted and published and it has been possible to quantify the mineral loss and gain. Although QLF has been expanded and developed for the quantification of smooth surface decay, the majority of lesions occur on the occlusal surface of the teeth. In order for a new technique to be useful to dentists there is a need to develop indices for mapping the measured values to a set of understandable relavant criteria to allow clinical decision making. Currently, an objective and a well-defined process for classifying carious lesions for all tooth surfaces by QLF does not exist. QLF is one of the approaches that has promise and potential in this area particularly as it may lead to a reduction in the number of X-rays taken so reducing exposure to radiation as well as decreasing the time required for examination and diagnosing.

QLF was observed to improve the identification of early demineralisation on tooth's surfaces and provide useful information regarding plaque red fluorescence in carious lesions whilst the Morita camera gave information regarding red fluorescence only. QLF was able to significantly differentiate all ICDAS II severity scores. A QLF Index was developed for all tooth surfaces *in vitro* to classify early carious lesions using ΔF at the 5% level. The index was further developed *in vivo* use for the occlusal, buccal and lingual surfaces of the tooth. Finally, a preliminary index to assess occlusal caries diagnosis and management was developed. Clinicians can rely on ΔF and ΔQ values to support decision making concerning the diagnosis and the degrees of early restorative intervention required. QLF technology may aid clinicians not only in early caries detection and classification but also to make a clinical diagnosis and to treat cases in more conservative way. It is anticipated that QLF will be valuable tool in routine dental clinical practice

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CHAPTER 1

INTRODUCTION

Dentistry as a profession appeared in the mid-nineteenth century focusing on the treatment of the oral diseases and problems which have bothered humans throughout history (Hoffman-Axthelm, 1981; Lufkin, 1938). Writings of the Egyptians, Mesopotamians, Indians, Chinese, Greeks, Romans, Aztecs, Incas and Arabs have documented reports and descriptions of tooth diseases and their treatment which was based simply on teeth extraction (Lufkin, 1938). In 1875 dentistry was experiencing a novel revolution that focused on preserving teeth rather than extraction (Lufkin, 1938).

One of the most common oral diseases in humans is dental caries (Loesche, 1986). Tooth surface is exceptional among other surfaces in the body in that it is a non shedding hard surface, which adsorbs mucins (acidic glycoproteins) from saliva, forming the acquired enamel pellicle (AEP) (Gibbons and Socransky, 1962; Lie, 1977).

This AEP is an amorphous membranous layer that encloses a high number of sulphate and carboxyl groups, this increases the negative charge of the tooth surface (Rogers, 1976). As the bacteria have also a net negative charge, then there is revulsion between the tooth surface and the bacteria. The defence mechanism breaks down when plaque formation take place (Loesche, 1986). Therefore, caries is a disease of bacterial origin, which starts in dental plaque (Fejerskov and Manji, 1990; Fejerskov and Thylstrup, 1994; Manji et al., 1991). Dental plaque has been defined as the diverse microbial community found on the tooth surface embedded in a matrix of polymers of bacterial and salivary origin (Marsh and Martin, 2009).

Dental tissues-enamel, dentine and cementum are the oral surfaces that are coated by pellicle to which microbial cells attach and colonise forming dental plaque (Kidd and Fejerskov, 2004). The two most important groups of acidogenic bacteria are the *mutans streptococci* and the *lactobacilli* (Loesche, 1986). These bacteria produce acids when they metabolise fermentable carbohydrates which can dissolve the calcium phosphate mineral of the dental hard tissues in a process known as demineralisation (Featherstone, 1990). In this process, the acids diffuse into the subsurfaces and produce hydrogen ions. These ions will lower the pH in the plaque and dissolve the minerals of the tooth structure. The principal constituent of tooth and bone mineral is mixed, poorly crystallised hydroxylapatite. Another calcium phosphate is octacalcium phosphate has a remarkable structural similarity (Brown, 1962). The chemistry of hydroxylapatite is greatly complicated by its ability to form interlayered mixtures with octacalcium phosphate (Brown et al., 1979). Dental mineral mainly consists of carbonated calcium hydroxyapatite which varies from calcium hydroxyapatite by the substitution of carbonate for a portion of phosphate in calcium hydroxyapatite. Carbonated calcium hydroxyapatite is more soluble than calcium hydroxyapatite especially in acidic media (Featherstone et al., 1990; Kautsky and Featherstone, 1993). Therefore, acids diffuse inwards and reaction products (calcium and phosphate) diffuse outward hence, caries is a disease of net mineral loss. All the acids produced by the bacteria- including acetic, formic, lactic and propionic acids- can readily dissolve the tooth minerals (Featherstone and Rodgers, 1981). Caries lesions are the signs of this oral disease. The site at which the caries occurs is determined by the acidogenic bacteria, the access to the pathological factors and the stagnation areas which encourage plaque

retention at that site. Demineralisation and remineralisation occur several times in the mouth everyday, with either progression or reversal of the process being the end result point (Featherstone, 2004). It is important to understand that the disease of dental caries includes a series of different sizes of lesions from microscopically subtle to clinically clear (Pitts, 1997).

Each tooth consists of a crown or coronal portion that extends into the oral cavity and is bathed by saliva, and a root portion that is attached by collagen fibres of the periodontal membrane to the jaw as shown in Figure 1.1. The crown of the tooth has five surfaces that have different tendencies for supporting plaque flora. The smooth surfaces are on the labial/buccal and lingual aspects of the tooth, the approximal surfaces and the occlusal surface, which is the chewing surface for molar and premolar teeth, are traversed by developmental grooves and fissures that are colonised by a scant flora and are the most liable to plaque formation relative to smooth and approximal surfaces.

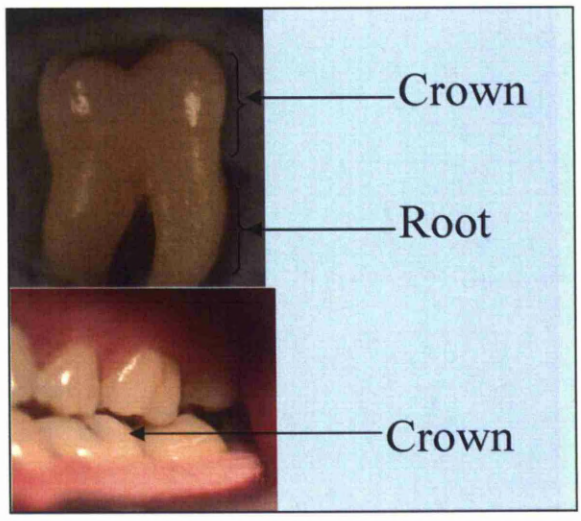

Figure 1.1: Tooth portions.

Caries lesions can be classified in different ways; according to their anatomical location, pit and fissures caries and smooth surface caries. Lesions may be initiated on enamel (enamel caries) and/or on exposed root cementum or dentine (root caries). Caries lesions can be classified according to the condition of the surface, for example if the lesion is on a previously sound surface; it is a primary lesion, whereas lesions adjacent to restorations are classified as recurrent or secondary caries. Caries left in place following cavity preparation at the time of restoration is classified as residual caries. On the other hand, caries lesions may be classified according to their activity, a progressive carious lesion (active lesions) and a lesion that formed earlier and then stopped (arrested lesion) (Fejerskov and Kidd, 2003).

Diagnosis is 'the art or act of identifying a disease from its signs and symptoms' (Merriam-Webster, 2003) and the diagnostic stage has been referred to as ' a mental resting place on the way of intervention' (Baelum and Fejerskov, 2003). In general, caries diagnosis tends to fall into three steps. Firstly the dental caries process is detected and described; secondly the signs and symptoms are explained and finally producing greater details using techniques such as bio-imaging. Many dissimilar understanding of caries are unhelpful when attempting to elucidate the process of diagnosis and improve dentist's clinical decision-making (Baelum et al., 2006).

In England and Wales in 1993, the decayed, missing and filled teeth (DMFT) in 12-year old decreased from 4.8 to 1.2 (O'Brien, 1993). In 1996, a review of dental caries prevalence in Europe conducted by Marthaler and colleagues showed that

more decline was going on (Marthaler et al., 1996). Trends over time show a modest improvement of 4% in overall DMFT in children for Britain since 1997/98, compared with the 8.6% improvement seen for the two earlier years. The care index has remained nearly unchanged in Britain as a whole 13.6% in 1999/2000, compared to 13.9% in 1997/1998 (Pitts et al., 2001).

Although a decrease in dental caries is good news, it is a diverse blessing in the dental world. As the overall caries experience declines, it does so in the least susceptible smooth surfaces whereas there is much less effect in the most susceptible pit and fissures surfaces. This is due to a number of factors for example the compound morphology of pits and fissures is considered to be a place for plaque, bacteria and food retention, making mechanical debridement unattainable. Other factors responsible for the high incidence of occlusal caries include lack of salivary access to these fissures. Salivary dysfunction caused by factors such as medications, radiation therapy for cancer of the head and neck, some systemic diseases, or genetically induced conditions. In addition other factors such as mouth breathing or anxiety may cause a dry mouth. In little children, medications such as anti-asthma therapy may cause hyposalivation, which is a major risk factor. Pediatricians, parents, caregivers, and health care professionals must be aware of the importance of medication-induced saliva flow reduction as a risk factor (Featherstone et al., 2003). Therefore, in populations with decreased caries prevalence, the propotion of occlusal caries has increased (Lussi, 1991; Mejare et al., 1998). Furthermore, the national surveys of Adult Dental Health have shown that, a larger proportion of the increasing elderly population are now retaining more

of their natural teeth for longer and the percentage of edentulous adults in England and Wales has reduced from a value of 20% in 1988 to 12% in 1998 (Walker and Cooper, 2000). As the proportion of the population who are dentate increase, the number of natural teeth at risk of developing dental caries also increases. In spite of the decrease in prevalence of dental caries, it is still a problem of great significance (Angnes et al., 2005). This has stimulated renewed interest in developing methods for early caries detection, minimal intervention and preventive strategies.

Carlos and Gittelsohn in 1965 suggested that teeth could be grouped according to the order of susceptibility from greatest to the least as follows: lower first and second molars, upper first molars; upper second molars; upper first premolars, upper and lower second premolars; upper incisors; upper canines, lower first premolars, lower incisors and lower canines. In a longitudinal study of young adults 14-25 years of age, it was found that occlusal surfaces on molars and premolars accounted for 60 % of the total decayed, missing and filled tooth surfaces (DMFS) score (Crossner and Unell, 1996). Over recent decades, it has been claimed that the reduction of dental caries prevalence rate in many European countries complicates the diagnosis of dental caries, especially on the occlusal surface of the teeth (Creanor et al., 1990; Kidd et al., 1993b; Kidd and Joyston-Bechal, 2005; Le et al., 1995; Sawle and Andlaw, 1988; Verdonschot et al., 1992; Weerheijm et al., 1989; Wenzel and Fejerskov, 1992).

In spite of the fact that fluoride is abundant and available in various forms, and plays a role in caries reduction; it has also been reported that fluoride has more

beneficial effects on smooth and proximal tooth surfaces and is less effective on the occlusal surfaces (Backer Dirks, 1974; Groeneveld et al., 1990). Backer Dirks and his colleagues showed that water fluoridation reduced caries (Backer Dirks et al., 1978). A study in 1988 has suggested that fluoride may be responsible for the change in the disease presentation (Swale and Andlaw, 1988) because it facilitates enamel remineralisation and/or slowing the progression of the disease. In addition it masks the spread of caries in dentine. On the other hand, it also afforded the dentists with the chance to diagnose and deal with dental caries at early stages (Ferreira Zandona, 2006). Different effects of fluoride in preventing and/or reducing dental caries on different tooth surfaces have been widely reported (Lussi, 1991; Newbrun, 1992; Pitts, 1997; Whelton, 2004).

The effect of fluoride on the occurrence of hidden caries remains unknown. However, fluoride alters the model of caries attack and the occlusal surfaces become of special significance (Weerheijm et al., 1997). Recently, clinicians face increasing difficulty in diagnosing occlusal caries because what appears to be clinically sound enamel on visual inspection may hide a large dentinal lesion "concealed caries" (Figure 1.2). These lesions have come to be called "fluoride caries" (Millman, 1984), "hidden caries" or "occult caries" (Ball, 1986). This type of caries was found in 10-50% of the teeth with dentinal caries (Creanor et al., 1990; Kidd et al., 1992; Weerheijm et al., 1992).

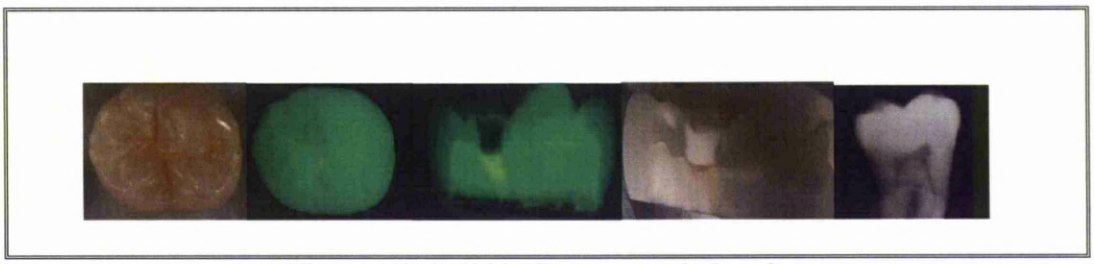

Figure 1.2: "Hidden" or concealed caries.

The occurrence of hidden occlusal dentine caries was reported by general practitioners (Kidd et al., 1993b) and has now been recognised by researchers (Lussi, 1993; Pitts, 1996a; Stedtler et al., 1996). Factors such as the presence of plaque, stains, and anatomical variations add to the difficulty of diagnosis. Studies in Dundee have ascertained that 42% of occlusal radiolucencies in dentine were scored sound by a trained examiner (Pitts and Deery, 1994). Changes in caries patterns can be summarised as:

1. Reduction of caries incidence mainly in children.
2. Remarkable changes in caries distribution and shape which has influenced by fluoride making cuspal enamel more resistant to fracture.
3. Slower progression rate.
4. Reduction in lesions developed on the smooth surfaces.
5. More caries found on the occlusal surfaces.

Several methods for dental caries diagnosis have been used for more than half a century including: visual, optical, tactile, radiographic, electrical and some emerging novel technologies. Visual inspection, the most widespread caries detection system, is subjective (Pretty et al., 2006). It is important to believe that

visual examination is constantly the first clinical stage in any type of currently available technology used in the diagnosis of dental caries. It helps in assessment of colour and texture which are qualitative and provide some information on the severity of the disease but fall short of true quantification and early detection of lesions (Maupome and Pretty, 2004).Visual examination for occlusal caries diagnosis has shown high specificity but low sensitivity and reproducibility (Bader et al., 2002; Costa et al., 2002; Ketley and Holt, 1993; Lussi et al., 1999). On the other hand, the use of a sharp probe with visual examination does not appear to improve the diagnostic truth (Penning et al., 1992), it may harm the fissures (Ekstrand et al., 1987) as well as it may make lesion progression possible (Van Dorp et al., 1988).

The data from a study conducted in 1979 showed that, the dental explorer might serve as a mean for intra-oral spread of *Streptococcus mutans* from tooth to tooth in a given mouth (Loesche et al., 1979). It is vital to note that the simple "bolt" of an explorer is not adequate to diagnose caries on the pits and fissures because a sharp dental explorer can be pushed into a healthy surface and be retained giving a false diagnosis. Therefore, the value of the dental explorer or dental probe in the detection of caries especially in the occlusal surfaces of the teeth has been questioned by investigators (Loesche et al., 1979 ; Lussi, 1991). More suitable tactics involve using the dental explorer to remove dental plaque and assess the tooth surface smoothly (Hamilton, 2005). Today, most of the clinical interventions are required on occlusal surfaces of the teeth, mainly in those with complicated fissure systems (Kidd et al., 1993b). In 1987 it was hypothesised that the occlusal

lesion is initiated on the fissure walls and concealed by the superficial sound hard dental tissues (Kidd and Joyston-Bechal, 2005). Progressive destruction of the occlusal surface is therefore initiated by a limited procedure either in the innermost part of the groove-fossa system due to the build up of bacterial deposits, or along the way in to deep fissures, or both (Fejerskov and Kidd, 2003).

At present, the very early demineralisation process is unnoticeable clinically or radiographically, so is likely to be missed by the clinician's diagnosing eye and the dental explorer. The first signs of dental caries (dull white spot) are often not present until the decalcification has progressed 200 to 300 μm into the enamel and the minimal depth of a detectable lesion on radiographs is about 500 μm (Beiswanger, 1996). The white-spot stage in the caries-prone fissures and approximal surfaces cannot be visualised directly during usual clinical dental examination (Loesche, 1986).

Since the discovery of X-rays by Wilhelm Conrad Roentgen in 1895, radiographs have been used to detect dental caries and its effects on the hard dental tissue. These are mainly used for the detection of dental lesions on the approximal surfaces, which are difficult to inspect clinically and also used as an aid for the detection of occlusal caries in clinical settings.

Radiographic diagnosis is based on the fact that a decrease of attenuation of the X-ray beam results from the decrease in mineral content that occurs in dental caries. Many factors can influence the accurate visualisation of caries lesions on

radiographs including: exposure parameters, type of image receptor, film processing, display method, viewing circumstances and the knowledge and skill of the clinicians. Systematic review of the literature points out that the power of evidence from radiographic means for caries detection is poor for lesions on posterior occlusal and approximal surfaces of the teeth. Despite this however, it does not indicate that the radiographs are of no diagnostic merit, but does suggest that the radiographs have a higher specificity than sensitivity. This means false negatives are more likely to occur in the presence of the disease than false positives in its absence. This may result in further progression of the disease and further loss or irreversible damage healthy tooth respectively (Dove, 2001).

In 1925 Raper introduced bitewing radiography as an adjunct to the visual-tactile dental caries examination. Bitewing radiographs show the depth of the lesion into the hard dental tissues, but the radiographic diagnosis is unreliable. The radiologic appearance of carious lesions is related to lesion depth with the probability of lesion detection varying with its true depth. The process of caries diagnosis cannot be predicted with certainty, even for deep lesions or intact surfaces (Baelum et al., 2006). Radiography is insensitive in early caries detection, as approximal caries lesions can be seen on the bitewings radiographs when they are at least halfway through the enamel histologically (Berg, 2007). The pattern of dental caries is shifting, with an increasing occurrence in occlusal surfaces. This change has made traditional diagnosing systems, mainly bitewing radiographs less helpful in the diagnostic protocols of clinicians (Pretty, 2006). Another challenge is the presence of radiopaque "islets" or areas within the radiolucencies demonstrating caries on

the bitewing radiographs as a result of mineralisation from fluoride (Pitts, 1984).This aspect, combined with the new, more grainy, lower contrast fast X-ray films makes radiographic diagnosis more demanding than in the past (Pitts, 1997). In 1997, it has been reported that bitewing radiographs could detect demineralisation in dentine but not in the enamel (Ricketts and Kidd, 1997). Additionally, in 1997 a study showed that radiographs were appropriate method to detect softened and unhealthy dentine, mainly in the middle or the inner thirds of the dentine but failed to detect in the outer third and in enamel (Ekstrand et al., 1997). Machiulskiene and his group have shown that radiolucency present in the radiographs does not provide information about the lesion activity, therefore, should not be used in isolation to inform the type of intervention to be used (Machiulskiene et al., 1999). On the other hand, there is a variation between dentists in radiographic caries diagnosis. A study done in 2001 showed that dentists produce many false-positive diagnosis of dentinal caries in approximal or occlusal surfaces whose actual state is either sound or enamel caries (Espelid and Tveit, 2001). Digital intra-oral radiographic systems seem to perform in a similar way to the currently available dental films for the detection of caries. In addition, it has been demonstrated that digital radiography is also of little value for the detection of early approximal and occlusal lesions (Castro et al., 2007; Wenzel, 1998).

Classically, dentists formulate their decision (absence or presence of dental caries) based on subjective signs and indications within the tooth such as colour and hardness which often, result in a large number of caries being missed (Ferreira

Zandona, 2006). It has been determined that conventional clinical methods used to detect occlusal caries in particular, result in low values for sensitivity (Lussi, 1993; Wenzel et al., 1991b) and the main drawback of the conventional methods is that they still rely on the dentists's subjective interpretation (Angnes et al., 2005). Variation in dental caries diagnosis and treatment decision is a recognised phenomenon (Bader and Shugars, 1995; Elderton and Nuttall, 1983; Kay and Knill-Jones, 1992). There is a significant difference between dentist in their clinical diagnosis and caries decision making (Baelum et al., 2006). Treating the results of tooth decay is what dentists spend most of their professional time undertaking. Over the years, dentists are treating the outcome of tooth decay but not the infection itself and most of the restorative, prosthodontics and endodontics dentistry is related to the outcomes of dental decay not to the disease itself (Berg, 2007). Historically, dentists are restricted to surgical restorative intervention due to the lack of clinical diagnostic tools that are sufficiently sensitive to detect caries at a stage where a therapeutic approach can be applied effectively to allow cessation of or reversal of caries process (Berg, 2007).

Over the past years the approach in dentistry has shifted from restorative handling of carious teeth towards prevention of new caries, arrest progression of the existing carious lesions or remineralisation of early caries lesions, and it is recognised that caries is probably avoidable and curable (Fried et al., 2007). In view of the fact that those small lesions can be remineralised relatively easily and more successfully than advanced lesions, it is important to identify and categorise early carious lesions so that minimal intervention can commence. Moreover, in the light of the shift in the direction of Evidence-Based Dentistry, which is looking to improve the

oral health care by making sure that the clinical decision, scientific judgment and the clinical practice are supported by current research knowledge.

Improper diagnosis may result in inappropriate treatment decisions, especially if the operative intervention is complicated. Incorrect restoration of teeth open a continuing restorative cycle because of the limited life of restorations, possible iatrogenic harm to adjacent teeth and cost in term of resources and oral health care. For these reasons full, comprehensive and careful examination should be emphasised. For this reason, the importance of diagnostic technologies is realised. Deficiencies among existing tools and methods have major implications if these allow false negative judgment of unseen occlusal dentinal lesions and approximal cavities on one hand, whilst generating some false positive judgment on sound surfaces leading to unsuitable decisions to restore on the other. Missed enamel lesions will not get targeted preventive care and may progress, while missed dentinal lesions will make the restorative intervention overdue which may lead to major tooth tissue loss or it may even be too late to save the pulp, which in turn will result in more complicated intervention that might end by restoration with greater chance of fracture and replacement. However, low specificities will generate the problem of unsuitable care (Pitts, 1997).

The working definitions of sensitivity and specificity according to Berg are, "sensitivity refers to the ability of the tool or device to identify the presence of the condition when it does indeed exist". "Specificity refers to the ability of the tool or a device to be accurate in its identification of a condition when it detects such condition"(Berg, 2007). However, if dentists and researchers have the ability to

apply the new caries detection tools, technologies and validate them clinically, databases will be generated which can be used to facilitate careful characterisation of caries lesions to permit systems to be engaged to enhance clinical decision making as to which lesions are likely to progress or may require longitudinal monitoring (Berg, 2007). These devices may detect but still require the clinician to make a diagnosis based on the data generated from the information out of different sources (Tinanoff, 2002).

Caries diagnosis is based upon the structured information collected from the patient's dental history and the results of a range of diagnostic procedures and filtered by the dentist's knowledge, skill and earlier experiences (Mileman et al., 1986; Mileman and Espelid, 1988). Consequently, dentists' features influence the caries diagnosis process such as experience, knowledge, ability, treatment, diagnostic methods, tools, and personal preferences, as well as equipments available, skills of personnel and how busy is the surgery. One of the shortcomings in caries management strategies is the lack of system which can reliably establish the extent of subsurface caries (Featherstone, 1996; Pine and Ten Bosch, 1996; Pitts, 1996b; Stookey, 2000).

For these reasons, it is important to proceed in dental caries diagnosis research to develop a diagnostic method and tool that gives the best outcome for dental patients (Baelum et al., 2006). One recent development proposed to decrease the subjectivity, increase the sensitivity when evaluating caries activity is The International Caries Detection and Assessment System (ICDAS) (Ekstrand et al.,

1997; Nyvad et al., 1999; Pitts and Stamm, 2004). Caries activity criteria used as part of ICDAS depend on the physical characteristics of tooth surfaces examined such as reflection, texture and colour of the lesion, with chalky rough surface being active, smooth shiny surfaces being inactive and arrested lesions have an internal brown colour and surface stain (Ferreira Zandona, 2006).

Novel Diagnostic systems are based upon the measurement of a physical signal which includes X-rays, visible light, laser light, electronic current, ultrasound, and possibly surface roughness (Verdonschot and Angmar-Mansson, 2003). All of these methods have limitations affecting either their diagnostic ability or their appropriateness in a clinical setting.

Therefore, there is a need for new methods that are synergising with the current detection system of visual assessment and X-rays (Ten Bosch, 1996). However, the most important factor will be the degree to which the diagnostic methods and lesion classifications bear on the dental health outcome. Therefore, contemporary approaches to the management of dental caries aim to include early prevention, control and intervention. This is based on a proper diagnosis of the disease, early detection of its pathological changes and clinical signs of tissue damage and its classification (Tranaeus et al., 2005). The caries diagnostic process is "a procedure during which observations are classified according to what is known about the aetiology, pathology, therapy, prevention and prognosis of each type of lesion observed and to be able to choose the most appropriate form of intervention to obtain the best health outcome for the patient in question" (Fejerskov and Kidd,

2003). Therefore, early and objective diagnosis represents a key milestone in new and advanced dental care and should be integrated to prevent dental caries and/or to act early. Moreover, the employment of intra-oral cameras in every day dental practice has significantly extended the range of tools to enhance the diagnostic decision-making and to motivate dental patients. This new technologies could provide health and financial advantages ranging from preventive treatment to facilitate clinical trails and research of dental caries.

1.1 Aims and Objectives of this thesis

The incidence of occlusal caries has increased and the aging population with their polypharmacies has caused a dramatic rise in dental caries. The diagnostic sciences have become one of the highest priorities in caries research.

To date there is limited data on QLF and dental caries. QLF parameters ΔQ, ΔF and ΔR mean little to dentists. Like any expanding technology, QLF presents new challenge for the specialist. Many *in vitro* studies and some *in vivo* studies had been conducted and published and it has been possible to quantify the mineral loss and gain. Whilst QLF has been expanded and developed for the quantification of smooth surface decay, the majority of lesions occur on the occlusal and proximal surfaces of the teeth. However, for this technique to be useful to dentists there is a need to develop indices for mapping the measured values to a set of regions for clinical decision making. Currently, an objective and a well-defined process for classifying carious lesions by QLF does not exist. QLF is one of the approaches that have promise with talented outlook in this area with regard to the constant aim

of reducing number of X-rays taken, radiation dose as well as the time required for examination and diagnosing.

The aims and objectives of the studies presented in this thesis were:

1. Develop an interpretative clinical index of QLF values for all tooth's surfaces.
2. To determine the cut-off points for different stages of the caries process by the use of QLF.
3. Validate *in vivo* the QLF occlusal caries index previously developed *in vitro* with additional information.
4. Evaluate and determine feasibility of QLF for the detection of approximal carious lesions.
5. Evaluate and determine feasibility QLF for the detection of "hidden caries".
6. To determine whether the QLF parameters were appropriate for aiding diagnosis and clinical decision making of early occlusal caries.

It was hypothesised that the respective diagnostic performances of visual examination, QLF, Morita-Penviewer, periapical radiographs and histology are the same.

QLF has been rising in popularity during the past years. In the future, QLF will offer several opportunities for routine clinical practice. Therefore, this thesis is focused on validation of the use of the QLF in the clinical settings and develops understandable and meaningful interpretative indices which will be applicable to the clinicians. This will support the use of QLF in every day practice.

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CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This review will discuss the nature, epidemiology of dental caries, together with its diagnosis and it will discuss some investigational test and techniques used to quantify tooth minerals.

2.2 An overview on dental caries

Dental caries is a chronic disease and it is a process which progresses very gradually in general in most human persons (Fejerskov and Kidd, 2003).
Dental caries is among the most prevalent unrelieved, non-infectious diseases of humanity (Larmas, 2010) with 91% of the adults population experiencing caries during their life (Beltrán-Aguilar et al., 2005). However, the wide spread use and application of fluoride has changed the nature of carious lesions by slowing disease, thus giving dentists the chance to diagnose and manage dental caries in its early stages, this in turn has led to an increase in the interest of the researchers and the experts to design and study new diagnostic devices (Ferreira Zandona, 2006). A "White-spot" lesion, which is the first visual clinical presentation of dental caries, does not actually represent an early stage of caries process. At this stage the porosity below the enamel surface has already increased, resulting in light scattering and loss of enamel translucency (Ferreira Zandona, 2006). The most challenging area in early caries diagnosis is trying to distinguish between active lesions, inactive lesions and the lesion which is in a transitional stage, either from active to inactive or vice versa. All these factors lead to uncertain diagnosis.

Caries activity is complex to diagnose comprising immediate past caries experience, lesion progression and the clinical appearance of the lesions (Shi et al., 2003). This presents some of the restrictions of the standard ways of dental caries diagnosis involving the lack of ability to detect early bacterial action inside the tooth or around/under the surface of dental restorations.

Accurate diagnosis of the presence, extent and activity of the caries process is a fundamental requirement, as this will help to avoid substantial overtreatment and precludes appropriate management which may compromise the long term tooth survival (Downer, 1989). The appropriate classification of occlusal caries lesions has become more difficult. This is one of the reasons behind the call for more reliable diagnostic tools over the past years (Pine and Ten Bosch, 1996; Pitts, 2001; Verdonschot et al., 1993a; Wenzel et al., 1991b).

Nowadays, caries diagnosis involves detecting of early signs of caries clinically and its activity (Featherstone, 1996). Accordingly, this can enhance the consistency of caries risk assessment. Hence, the appropriate preventive, restorative or a combination intervention can be determined. The ideal caries diagnosis method and tool should be able to capture the caries stages from the earliest present and it should be precise, meaningful, easy to handle and practical for all surfaces with or without restorations (Beiswanger, 1996). For a century, loss of minerals from the hard dental tissues has been known to change the optical properties. It has been known that, under certain conditions, teeth will fluoresce, with the first trial using an ultra-violet (UV) light source (Stubel, 1911). Hence, this phenomenon had been

proposed as a useful tool for dental caries diagnosis (Benedict, 1929). Several methods have been developed to improve diagnose caries diagnosis (Lussi and Hellwig, 2006). Recent advances and major improvements in technology have been made to allow detection, quantification of early stages of dental caries; as a result different lesion stages can be more precisely identified. Special consideration given to the improvement of the visual method may provide evidence to detect mineral changes within the tooth surfaces and to categorise the level of progress and the required interventions. In the 1980s, Swedish scientists reported on laser autofluorescence of enamel, and then investigational efforts began in this area (Bjelkhagen and Sundstrom, 1981; Bjelkhagen et al., 1982; Sundstrom et al., 1985). On the other hand, in 1987 and 1988 Pitts and Longbottom explored the use of endoscopically viewed filtered fluorescence (EFF) for clinical diagnosis of carious lesions (Longbottom and Pitts, 1988; Pitts and Longbottom, 1987). This work developed in 1991 to include the use of intra-oral video system for caries detection, the prototype "videoscope" (Pitts, 1991) subsequently a new method related to fluorescence attracted considerable interest this method was termed Quantitative Laser Fluorescence. In 1992, Hafström-Björkman and his colleagues reported on use of laser fluorescence for the quantification of enamel demineralisation (Hafstrom-Bjorkman et al., 1992). In 1994, the theory explaining fluorescence effects has developed and a prototype clinical device based on the innovative concept, called Quantitative Light-induced Fluorescence (QLF) was launched (De Josselin de Jong et al., 1995) and later correlation between fluorescence and mineral loss has recognised (Ten Cate et al., 1996).

2.3 Light Transmission through Teeth

Sound enamel consists mainly of hydroxyapatite crystals which are tightly packed, giving the enamel a glass-like translucent look. The yellow-white colour of teeth is the effect of dentine shining through usually the transparent enamel layer. Light that penetrates the tooth will be absorbed or scattered inside the tooth and these properties are usually explained using photons theory. Absorption is " the process in which photons lose their energy, mostly by conversion to heat" while scattering is "the process in which the direction of the photons is changed without the loss of energy" (Fejerskov and Kidd, 2003). In sound teeth, scattering is more likely than absorption and in dentine both scattering and absorption take place more often along the light path than either occurs in enamel (Ten Bosch, 1996). In the lesion, increased porosity results in increased difference in reflective index, this in turn causes stronger scattering in the lesion than in the sound enamel. This leads to shorter light path in the lesion and the absorption per unit of volume is much smaller in the lesion, which results in weaker fluorescence (Ten Bosch, 2000). Therefore, in white spot caries lesions, scattering is stronger than in sound enamel as a result of the increase in changing direction of photons and back-scattering before they reach dentine which causes the light path in the lesion to be shorter; and the fluorescence is less strong (Fejerskov and Kidd, 2003). Consequently, new technology places more stress on the objective measurement of the light properties e.g. scattering, reflection, absorption and fluorescence (Hall and Girkin, 2004). Fluorescence is defined as the emission of light after preceding excitement. The fluorescence of natural hard tissues is primarily caused by dentine (Kuhnisch et al., 2002).

2.4 Quantitative light-induced fluorescence (QLF)

QLF (Inspektor Research Systems BV, Amestrdam, The Netherlands) is one of the newer technologies in the field; it is a novel technique developed for the detection of early carious lesions. QLF is one way of assessing light interactions with the dental tissues which require measuring and recording the emitted light from these tissues and will evaluate the quantity of mineral loss from the suspected area(s) relative to the encircling tooth structure. QLF technology makes use of light from a special arc lamp (Philips bv, Eindhoven, The Netherlands) within the visible frequency to induce fluorescence, and scan the entire tooth surface, rather than at a single point as with other methods.

Formerly, laser light was used to fluoresce teeth in a sensitive, harmless way for the detection of demineralisation and dental caries (Alfano and Yao, 1981; Bjelkhagen et al., 1982).

The QLF uses visual and harmless blue/violet light, with an excitation of 405nm. To ensure only the fluorescent light is detected and no ambient light from the original source is collected a low cut-off filter at $\lambda > 520$nm (Philips bv, Eindhoven, The Netherlands) is used in front of an intraoral charge coupled device (CCD) camera lens leaving green and red parts of the spectrum (De Josselin de Jong et al., 1995). By means of a frame grabber, digital live images are displayed real time on a computer screen and are stored on a QLF computer Figure 2.1.

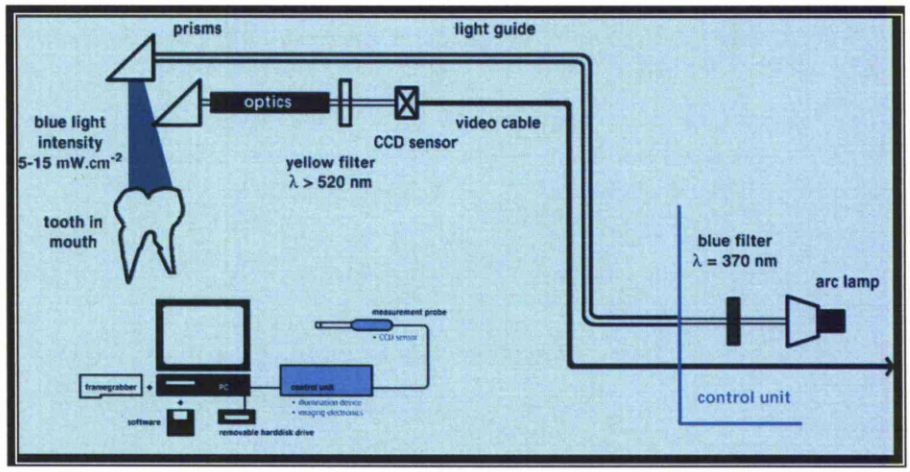

Figure 2.1: Diagram representing the QLF system (Courtesy of Inspector Research Systems BV, The Netherlands).

An image that is comprised of only green and red channels (the blue having been filtered out) is produced and the predominant colour of the sound enamel is green and it is called green fluorescence (GF) (Ando et al., 1997; De Josselin de Jong et al., 1995). Green fluorescence is an intrinsic possessions of hard dental tissues, it is the first type of fluorescence observed and is used to spot caries in its very early stages due to GF loss, and the red fluorescence (RF) in and on the teeth.

Since the initial attempts at *in vivo* quantification of mineral changes in the enamel the QLF method has continued to undergo further development, with respect to both software and hardware (De Josselin de Jong et al., 1995; De Josselin de Jong et al., 2009). In the last version of QLF software, the lesion area (mm^2), ΔF (average change in fluorescence, in %), and ΔQ ($\Delta F \times$area, %. mm^2) were the parameters. Therefore, it quantifies the lesion size, depth and volume from the image of the tooth using this software. To facilitate clinical use, a promising new

tool, the portable fluorescence device (Inspektor Research System BV, Amsterdam, The Netherlands), has been developed with new light source and filter system which replaced the laser light (Al-Khateeb et al., 1997). In this device a 35-Watt Xenon microdischarge arc lamp which produces 13 mW/cm^2 violet/blue light used as a light source. The liquid filled light guide transports the light to the tooth, then the emitted light of the tooth is collected via a special dental mirror by a video camera (De Josselin de Jong et al., 1995; Heinrich-Weltzien et al., 2003).

QLF uses the intrinsic fluorescence of the teeth. The fluorescence radiance of a carious lesion is lower than that of the sound hard dental tissues because of the consumption of fluid into pores resulted from demineralisation together with the uptake of stain, bacteria products. Caries will alter the regular interaction of light with the dental tissues. This cause more scattering of the light from the carious tooth tissues, and no or little fluorescence is noticed and it will be shown as a dark area(s). This phenomenon might be completely described by light scattering effects, even though a loss of fluorescing chromophores from a lesion can not be excluded as a causative issue (Ten Bosch, 1996). The cause of the auto-fluorescence is thought to be the enamel dentinal junction (EDJ) as the excitation light passes through the transparent enamel and excites fluorophores contained within the EDJ (De Josselin de Jong et al., 2009) (Figure 2.2).

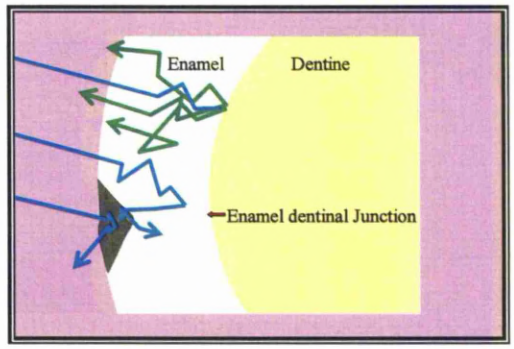

Figure 2.2: The cause of autofluorescence (Modified from Inspector Dental Care, Amsterdam, The Netherlands).

Green fluorescence is thought to be caused by intrinsic fluorophores inside dentine structure, mainly around the EDJ (Angmar-Mansson and Ten Bosch, 2001). This has been established in a number of experiments. In 2002 Amaechi and Higham incrementally removed dentine and measured the emitted light. They found that there was no impact upon the fluorescence until the EDJ was removed then, there is a noticeable drop in the emitted light (Amaechi and Higham, 2002). Another study conducted in 2003 has shown that as the enamel thickness decreases, fluorescence increases, as the EDJ is nearer to the excitation source (Ando et al., 2003). It was found that the area of strong fluorescence represents a thick band, rather than a narrow line represented by EDJ. The reason behind that was *Mantle* dentine. *Mantle* dentine is the first component of dentine to form immediately below the enamel and it is of 20 μm (Ten Cate, 1978; Ten Cate, 1998). The impact of *Mantle* dentine on optical properties was because that *Mantle* dentine differs from circumpulpal dentine in:

1. The orientation of the collagen fibres is at right angles to the EDJ in *mantle* dentine rather than parallel in circumpulpal dentine.
2. The collagen fibres are larger in diameter in *mantle* dentine.
3. *Mantle* dentine is hypomineralised.
4. The proteins within the *mantle* dentine are non-phosphorylated and mineralisation process differs, being vesicle mediated rather than matrix mediated.

Therefore, the structure may offer a reservoir of intrinsic proteinous fluorophores responsible for the auto-fluorescence of teeth (Ten Cate, 1998).

From the time when QLF has first introduced, the technology has continued to be developed so that it is now recognised as an intra-oral method that can be used for the detection and monitoring of caries, plaque and calculus.

QLF is a quantitative diagnostic method which presents a number of potential advantages over conventional visual examination techniques, which are summarised in Figure 2.3.

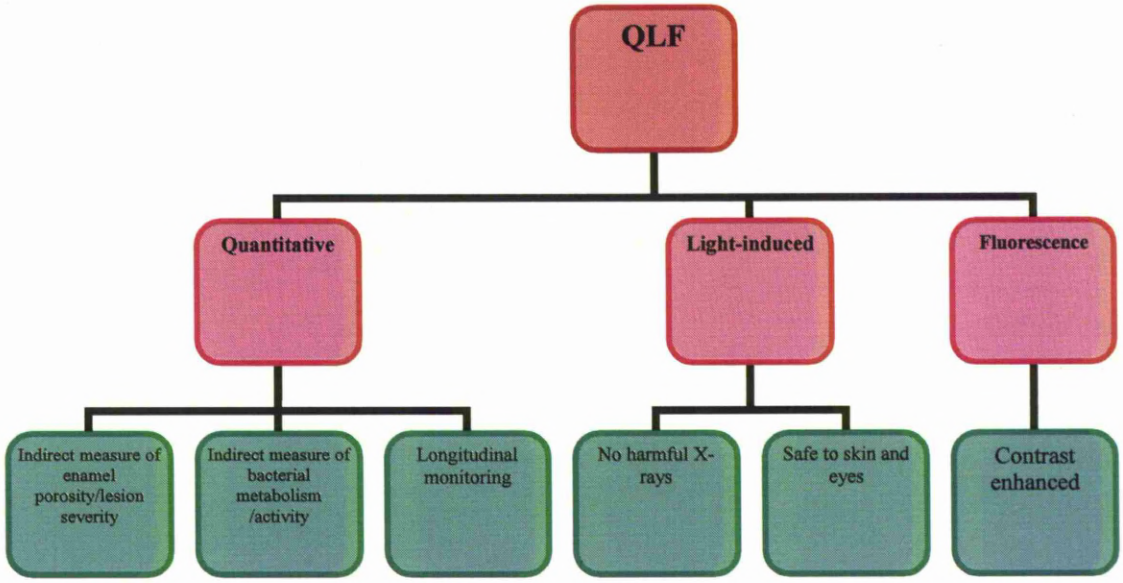

Figure 2.3: Potential advantages of QLF, modified from (Van der Veen et al., 2003).

QLF is non-destructive, non-invasive, non-ionizing, uncomplicated to master and offers the dental clinicians and researchers a quantifiable research tool which produces archivable data. The major advantage is QLF has the custom made software that can provide a very detailed analysis by gathering component that captured information from the fluoresced tooth and use this to compute demineralisation. QLF is a diagnostic tool which is used to help in decision making about the most appropriate clinical intervention. It can also detect the tooth surface changes in less time than normal visual examinations (Waller et al., 2003). QLF has fewer limitations than many other caries detection methods since it combines the sensitivity and specificity as well as being less time consuming *in vitro* and additionally has the advantage of being as clinically useful as many methods used *in vivo* (Al-Khateeb et al., 1997).

In 1987 Angmar-Mansson and Ten Bosch found that there was indirect relation between porosity of carious lesions and the reflective index, as the former increased the latter decreased (Angmar-Mansson and Ten Bosch, 1987). This theory was used to develop a workable tool by taking images on CCD cameras (Hafstrom-Bjorkman et al., 1992). QLF afford further visual data about tooth decay as it enhances the contrast differences between healthy and diseased tooth tissue by the principle of fluorescence (Waller et al., 2003). In a sound tooth structure, green fluorescing light will be shown as the tooth absorbs the illuminated violet-blue light. Decreased and or absence of fluorescence relate to increased scattering as a result of changes in the mineral contents of the hard tooth tissues. In cases of early caries lesions, this will appears as a dark spot rather than white spot under the normal routine clinical examination conditions. QLF is two to ten fold higher in lesion detection compared with the visual examination, visual –tactile or visual examination together with radiographs and this varies according to the type of the tooth surface (Ferreira Zandona et al., 2000; Heinrich-Weltzien et al., 2003). With the addition of specialised QLF software, the level of fluorescence of dental tissue can be quantified (De Josselin de Jong et al., 1995). The system consists of a handpiece attached to the system box through a cable which houses both the light guide and the video wire. The Inspektor™ Pro specification presented in Table 2.1. The system box is placed on a wheeled table together with a computer, monitor and wireless keyboard and mouse as shown in Figure 2.4. In this computer QLF software (QLF version 2.0.0.39, Inspektor Research Systems BV, Amestrdam, The Netherlands) was installed.

Table 2.1: The Inspektor™ Pro Specification.

Inspektor™ Pro	
Dimensions (w x d x h)	641 x 596 x 946 mm.
Weight (total)	40 kg.
System Box	
Dimensions	400 x 340 x 81 mm.
Weight	7.5 kg.
Length of light-guide	2500 mm (98.4 ", 8.2 ')
Computer	
CPU	Pentium® IV
Weight (without accessories)	7.3 kg (16.1 lbs)
Software	Microsoft Windows XP Professional SP2 Inspektor™ Pro Software Inspektor™ Pro Installation and information CD Inspektor™ Pro Recovery Disk
Accessories	Cherry compact keyboard Mouse 15" LCD color monitor Footswitch (2-pedals)
Trolley	
Dimensions (unfolded)	641 x 596 x 946 mm (25.2 x 23.5 x 37.2 ")
Weight (total)	20 kg (44.1 lbs)

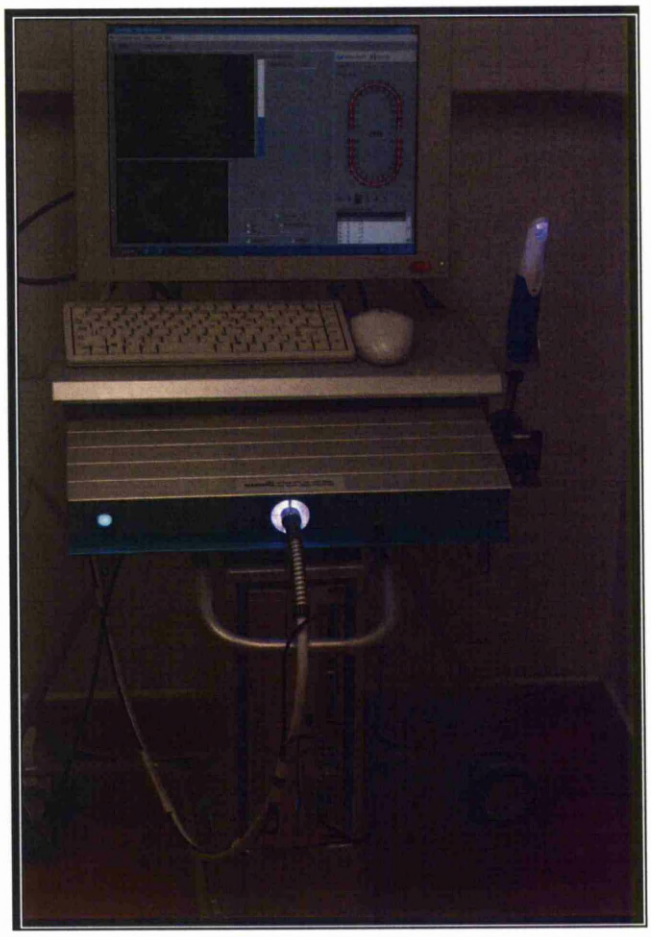

Figure 2.4: The Inspektor™ Pro.

In order to make this system compatible with clinical conditions and circumstances, to facilitate its usage by the clinician without the need of extra help from the dental assistant and to help in infection control as well, a foot paddle was introduced (Figure 2.5) Which is connected to the device by means of (Universal serial Bus) USB. It just need a light touch to capture the image and another one to allow saving of that image on the QLF Personal computer (QLF PC).

Figure 2.5: The Inspektor™ Pro foot paddle.

In vivo, QLF can image all tooth surfaces apart from inter-proximal site in fully dentate subjects. Live images are shown via a computer and accompanying software allows patient's details to be entered and images of the tooth surface of interest to be captured and stored (Figure 2.6).

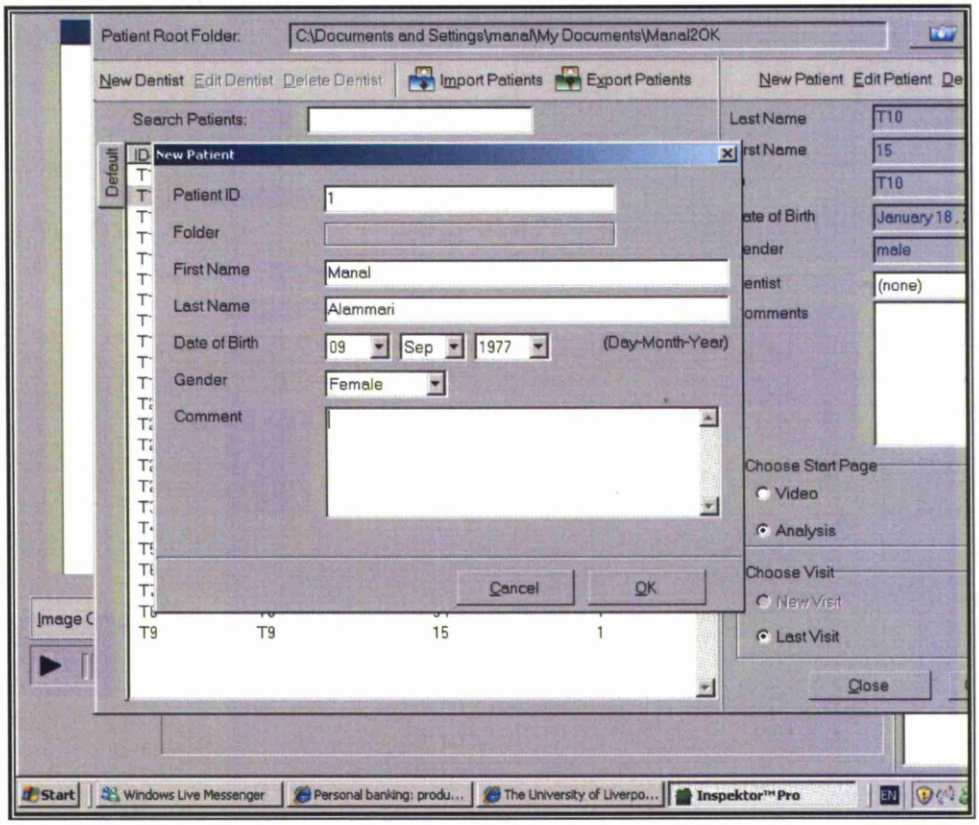

Figure 2.6: Image representing the new patient data entry in QLF software.

Although QLF was developed to recognise carious lesions, it was observed that, some QLF images showed areas on and/or within the tooth with red fluorescence of varying hues (Figure 2.7).

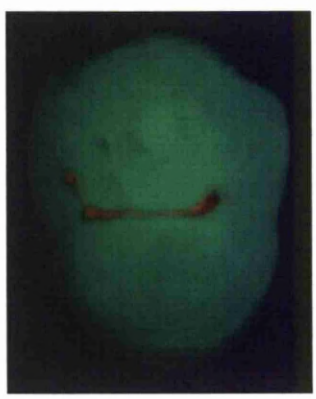

Figure 2.7: QLF image on occlusal surface of the tooth shows red fluorescence.

The phenomenon of red fluorescence in carious dental hard tissue and in dental plaque and calculus was first reported in the 1920s (Benedict, 1928; Bommer, 1927; Van den Bergh, 1928).

Studies have subsequently shown that these red areas are caused by bacterial biosynthesis like porphyrins mainly protoporphyrin IX, the accurate nature of the fluorescing chromophores is still unidentified (König et al., 1993). Red fluorescence which has been detected in QLF images has been supposed to be associated with caries risk (Heinrich-Weltzien et al., 2003). Then in 2005, it has been proposed that plaque associated with caries demonstrates red fluorescence (Izu et al., 2005). In 2006 a study has concluded that plaque at the ginigival margin and the stagnation area shows red fluorescence and it showed that in mature plaque, *Prevotella micros* fluoresce powerfully when it in proximity of *Prevotella gingivalis* (Van der Veen et al., 2006).

Sometimes, the red fluorescence can be removed from the tooth by careful professional cleaning. On the other hand, red fluorescence may persist even after cleaning (Waller et al., 2003). Furthermore, red fluorescence is found in more advanced lesions (dentinal lesions) and progressive white spots and in aged plaque as well as in calculus (Van der Veen et al., 2003).

2.4.1 Facts and Evidence

Pretty and colleagues summarised the predictive values in a high-risk and a low-risk group. They found that the positive predictive values vary between 0.90 and 0.98 and the negative predictive values vary between 0.35 and 0.70 according to the surface and lesion type. Consequently, QLF would indicate that the dentist could be more than 90% sure that the tooth was carious in a high-risk group (Pretty et al., 2003d). On the other hand, there are few *in vivo* studies applying QLF (Al-Khateeb et al., 1998; De Josselin de Jong et al., 1995; Ferreira Zandona et al., 2000; Tranaeus et al., 2001a; Tranaeus et al., 2002). Table 2.2 summarises the evidence supporting the use of QLF to detect caries, both the initial validation studies and more recent work to ascertain sensitivity and specificity. More clinical studies are needed to provide more expansive assessment of the QLF device.

Table 2.2: Summary of studies assessing quantitative light-induced fluorescence device. Modified from (Pretty and Maupomé, 2004).

SUBJECT	STUDY	THE MAIN FINDINGS
Occlusal caries	(Pretty et al., 2003c)	Strong correlation with histological findings (0.82).
	(Ando et al., 1999)	The correlations of TMR ΔZ to QLF 0.83.
	(Ten Cate et al., 2000)	Sensitivity 0.77, specificity 0.71
Reliability	(Tranaeus et al., 2002)	Intra-observer agreement 0.96, inter-observer agreement 0.97.
Secondary caries	(DeSchepper et al., 1996)	Sensitivity 0.88, specificity 0.85.
	(Benedict et al., 1996)	Sensitivity 0.95, specificity 0.85.
Root caries	(Pretty et al., 2003b)	Correlation with gold standard 0.89.
	(Gonzalez-Cabezas et al., 2001).	Receiver operating characteristic value of QLF technique 0.78.
Smooth surface caries	(Hall et al., 1996).	Sensitivity 0.75, specificity 0.90.
	(Shi et al., 2001)	Sensitivity 0.76, specificity 0.92.

Approximal caries lesions are usually hard to spot clinically, because they are not so accessable. Progress has been made with the development of quantitative caries detection techniques. However, none of these techniques has been applied successfully interproximally (Buchalla et al., 2002). To date no caries diagnostic technique is available which can be applied approximally and gives quantitative results at the same time.

In summary QLF appears to be a method that:

1. Sensitive for caries evaluation.
2. Enables discovery of caries lesion at a very early stage.
3. Allow lesion development monitoring.
4. Assess progression, regression or constancy.
5. Store images which can be sent out easily for referral and/or consultation.
6. Offers potential tool for clinical research by reducing the required time and improving the subject compliance.
7. Improve inter-examiner reliability.
8. Reduce the required time to prepare and calibrate examiners.
9. Allows more precise statistical methods for data analysis (Tranaeus et al., 2001b).
10. Its intra-examiner and inter-examiner agreement analysis is high. Any important differences detected between QLF examinations can be attributed to the experiment rather than examiner error.
11. Provide a user-friendly and reproducible technique (Pretty et al., 2002b).

12. Safe for frequent patient's examination.
13. Safe to the clinicians and the dental team as no radiation produced.

QLF has been reported to be a valuable tool for the detection and quantification of dental caries (Angmar-Mansson and Ten Bosch, 2001; Tranaeus et al., 2001a). Additionally, histological studies proved that loss of fluorescence measured by QLF is an excellent parameter for mineral loss expression in carious lesions (Ando et al., 1997).With the application of QLF, numerical values or a range of values have been assigned to the degree of fluorescence, which in turn may be used as an indicator of the degree and severity of caries i.e. QLF index.

2.5 Penviewer- Morita Intra oral Camera

This device has two handpieces. The first one is known as handpiece W and has four white LEDs, and the second is handpiece B with four blue LEDs and 2 white LEDs. Both can be used at the same time, and can be easily operated using a single control box Figure 2.8. It is a compact and light instrument, with a 13mm head which is small and simple to move around inside the oral cavity Figure 2.9. The camera has an excellent depth of field which eliminates the need for focusing. A video cable is used to transmit the camera's output to any TV monitor.

Figure 2.8: Penviewer-Morita Intra oral Camera.

Figure 2.9: Morita camera handpiece B.

2.6 Gold standards for the entire caries lesion process

Three Universal Criteria should be fulfilled for the development of new devices techniques are:

1. Have to be reproducible.
2. Have to reflect the patho-anatomical look of the disease under investigation.
3. Have to be independent of the diagnostic test under evaluation (Wenzel et al., 1991a; Wulff, 1981).

A list of *in vitro* caries diagnostic studies published between 1996 and 1999 shows that stereomicroscopic inspection of tooth sections (histology) is the most appropriate technique for a gold standards Table 2.3. This technique has been used from the earliest studies of diagnostic research (Downer, 1975). Jointly with transverse microradiography (TMR), it has been used to look at the nature and the progression of caries lesions (Mortimer, 1964). Microscopic examination is based on visual changes in the tissues. For enamel, a reduced mineral content is observed as opacity but the detection of demineralised dentine is less straightforward (Huysmans et al., 1998) and it is usually defined as "discoloration" (Ashley et al., 1998; Ekstrand et al., 1997). The disadvantages of the histological validation method are, however, that it requires considerable economic, resources, time and can be performed only in extracted teeth because of its destruction nature (Hintze and Wenzel, 2002).

Table 2.3: Summary of studies showed that stereomicroscopic inspection (histology) is the most appropriate technique for a gold standard.

Author (year).
(Hintze et al., 1995).
(Ekstrand et al., 1997).
(Ricketts et al., 1997).
(Tyndall et al., 1997).
(Ashley et al., 1998).
(Ekstrand et al., 1998).
(Ferreira Zandona et al., 1998a).
(Lussi et al., 1999).
(Wenzel and Hintze, 1999).

2.7 Transverse Microradiography (TMR)

For the quantification of the mineral loss, TMR is used. It is the most practical and widely accepted method used to assess de- and re-mineralisation in dental hard

tissues in *in situ* and *in vitro* studies. It is a highly sensitive method to measure the morphology and the change in mineral content of enamel and dentine samples. It is the analytical method which yields the most thorough quantitative information to date (Arends and Ten Bosch, 1992). However, it requires the destruction of the sample and can only be employed after completion of the experiment. It was first employed in the 1960's in dental research (Angmar et al., 1963). TMR was demonstrated by De josselin de Jong and his collaborators to measure mineral loss in caries research (De Josselin de Jong et al., 1987).

The technique has been constantly developed and improved. The developments in computer-aided video-image analysis of microradiographs have made TMR a suitable tool for determining small changes in mineral density profiles in time. Moreover, because many research groups have acquired TMR methodology and knowledge, it has become a standard method, or a 'gold standard', by which other recently developed methods, especially those designed for clinical diagnosis of caries, are compared and validated.

2.8 Microcomputed Tomography (μCT)

In cariology research there is an increased demand for non-destructive techniques of mineral change analyses. Not only do they considerably simplify investigative procedures in the laboratory (Hafstrom-Bjorkman et al., 1992), but allow longitudinal experiments to be conducted, once samples are preserved and can be analysed after different procedures in the same study, enabling evaluation of mineral loss, gain and its kinetics (Dowker et al., 1999). In 1991, Ten Bosch &

Angmar-Mansson in a detailed review of quantitative methods for mineral changes analysis recommended the development of a radiographic method to quantify mineral loss from whole teeth (Ten Bosch and Angmar-Mansson, 1991). The interest in radiation techniques is due to the ability of X-ray to travel through matter (Bonse and Busch, 1996; Herkstroter et al., 1990), for non-destructive evaluation and testing of objects (Zolfaghari, 1996).

X-ray Microcomputed tomography (μCT), used to non-destructively measure the mineral concentration in calcified tissues, and was validated for use in dental tissues (Amaechi, 2004; Amaechi and Saldaña, 2004; Amaechi, 2005; Dowker et al., 2004). It has the benefit of being a non-destructive technique for obtaining quantitative measurements of mineral concentrations within bulk specimens from the linear attenuation coefficient. Although it is still only appropriate for extracted teeth, mineral concentration can be determined from the linear attenuation coefficient with an error of <0.2 g cm^{-3}. The resolution of μCT can be sufficiently high (~1 μm) as to provide a relation between the understanding of structural changes observed by electron microscopic studies and the knowledge of larger-scale changes in mineral distribution determined quantitatively in many microradiographic studies. It collects closely spaced parallel slice images through the sample, and presents both qualitative and quantitative assessments of the sample in both 2-D and 3-D images.

The huge advantage of this system is its non-destructive nature which enables the assessment of caries activities *in vitro* over time and specific sample preparation is

not required, on the other hand scanning may take an extended period of time and this may be a limiting factor, to date this relatively new technology has been used in a number of *in vitro* evaluations of the dental hard tissues, dental caries and restorative procedures

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CHAPTER 3

Development of Caries Indices Using Quantitative Light-induced Fluorescence (QLF) *in vitro.*

3.1 Introduction

Teeth have many important roles, for example in chewing, talking, pronunciation and aesthetics, the latter is often perceived to be of major importance. Furthermore, many patients are unwilling to accept loss of their teeth (Walmsley et al., 2007). Therefore, early detection and objective diagnosis of caries are a major goal in modern restorative dentistry.

Over the past 30 years, a downturn in the rate of dental caries progression has been observed in many European countries (Marthaler, 1990) with the possible remineralisation of initial lesions. Alteration in diet and/or consumption of diary products (Higham et al., 1991), modification in oral hygiene in combination with widespread availability of fluoride may halt the progression of the lesion and in favourable conditions may even allow its remineralisation. This is possible if the demineralised dental tissues are not at the cavitation stage. Generally by the time carious lesions are detected clinically, they are beyond the stage where remineralisation is possible and restoration is inevitable (Hibst et al., 2001). Prerequisites for promoting remineralisation within a preventive management strategy include the early detection of the dental decay process together with reproducible, longitudinal monitoring of lesion which permits evaluation of the effectiveness of prevention. Together, these provide clinicians with more information to aid clinical diagnosis making with regard to whether treatment needs to be operative or preventive.

In recent years there have been attempts to improve the reliability of traditional detection methods. New techniques for caries detection and quantification have also been developed. One recent development is Quantitative Light-induced Fluorescence (QLF) (De Josselin de Jong et al., 1995) in which changes in the tooth substance associated with mineral loss and progression of the carious process is reflected in the alteration of fluorescent properties of enamel. Many *in vitro* studies have been performed to recognise the most favourable conditions which must be adhered to when using the QLF. These included investigations of the consequence of dehydration, presence of stains and plaque, lightening conditions and focal distance (Pretty et al., 2002a; Pretty et al., 2004). However, the available data is limited especially for occlusal caries. The validity of caries lesion quantification with QLF for smooth surface caries, has been the topic of many scientific papers (Al-Khateeb et al., 1997; Emami et al., 1996; Hall et al., 1997; Lagerweij et al., 1999). Validation studies have been performed and reviewed recently (Pretty et al., 2003a). The validity and reproducibility of the method require independent investigation, and the accuracy compared with other methods of detection if the technique is to be adapted for clinical practice.

In vitro trials are the most frequently applied techniques in dental caries research. *In vitro* methods have a number of advantages. They avoid ethical dilemmas connected with the use of human or animal subjects, they allow specific variables to be selected and tested, they allow uniform testing of techniques and/or preventive products and offer the opportunity of the use of greater number of methods to examine and quantify lesions (White, 1995).

This study uses QLF to develop indices to identify caries in extracted human teeth. Harmless, visible blue light is used to illuminate the teeth. This method has been widely tested, improved, and used in the dental research world but indices have not been available for dental professionals until now for all tooth surfaces to aid clinical diagnosis.

3.2 Aim and objectives

3.2.1 Aim

The overall aim of this study was to develop and validate an appropriate index for QLF by using QLF to quantify *in vitro* the extent of mineral loss in caries lesions affecting all tooth surfaces.

3.2.2 Objectives

1- Relate green fluorescence loss and level of red fluorescence to International Caries Detection and Assessment System scores (ICDAS II) and other techniques including histology, TMR, μCT and radiographic examination for all tooth surfaces (occlusal, buccal, lingual, mesial and distal).

2- Validate the previous occlusal index developed *in vitro* (Higham et al., 2003) by using a larger sample and by the application of more advanced technique and recent visual index.

3- Evaluate the use of QLF *in vitro* for the detection of approximal carious lesions.

4- To apply the use of QLF *in vitro* for hidden caries diagnosis.

3.3 Material and methods

3.3.1 Sample size

A sample size of 100 teeth was selected. In a previous study conducted only on occlusal surfaces (Higham et al., 2003) a sample size of 75 teeth was used. In this study, because the aim was to develop an index for all tooth surfaces, a larger sample size was chosen.

3.3.2 Teeth selection and preparation

Freshly extracted human premolars and molars (n=100), covering the caries range from sound to grossly cavitated, were collected from patients undergoing extractions at King Saud University Dental Hospital in The Kingdom of Saudi Arabia and from patients undergoing extractions at The University of Liverpool Dental Hospital in The United Kingdom after consenting. Each tooth was thoroughly cleaned with pumice (Associated Dental Products LTD, dental material manufacturers, Purton, Swindon, Wilts), wet-and-dry paper (grit size of 400 μm) (Mirka, UK) and a handpiece (KaVo EWL) with brush (Minerva Dental Limited, Cardiff, UK) (Figure 3.1). Each tooth was assigned a code number which was applied throughout the study. The teeth were stored in distilled water with thymol crystals (0.1% w/v) (GPR ^{TM} , Poole, England) and kept in a dark, cold room at

6°C until analysed (Figure 3.2). Requirements of the Human Tissue Act (HTA) were met in a co-ordinated and standard manner (HTA, 2004).

Figure 3.1: Teeth cleaning apparatus.

Figure 3.2: Teeth coded and stored in distilled water with Thymol crystals.

3.3.3 Visual examination and scoring

The teeth were stored in water throughout the study other than for the examinations, when they were dried with a 3-in-1 syringe for 5 seconds. ICDAS II

visual scoring system was used for every surface for each tooth (Table 3.1) (Ismail et al., 2007). To facilitate this, each tooth surface was carefully examined using 2x magnification and air-drying. A value was assigned and the procedure was repeated twice two weeks after the first examination. A third examination was carried out if there were disagreements between the first and second examination.

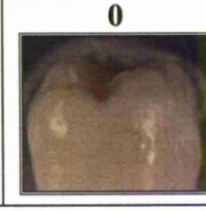
Table 3.1: International Caries Detection and Assessment System II scores and corresponding dental description.

DENTAL TERM	SCORE
Sound tooth surface.	0
First visual change in enamel.	1
Distinct visual change in enamel.	2
Microcavitation, localised enamel breakdown.	3
Underlying dark shadow from dentine with or without cavitation.	4
Distinct cavity with visible dentine.	5
Extensive distinct cavity with visible dentine.	6

From this study, a visual code system (VCS) was developed by the use of ICDAS II; visual assessment resulted in a 5 digit score of visual code system (VCS) for each tooth consisting of the single digit ICDAS II scores for each surface. As the

number increases, the severity of dental caries increases. Examples of teeth classified using the ICDAS II and VCS are shown in Table 3.2.

Table 3.2: Examples of teeth classified using the ICDAS II and the visual code system.

Tooth Study Code.	Visual Examination Index.				
	Occlusal	Bucal	Lingual	Mesial	Distal
T40	**6** 	**6** 	**2** 	**3** 	**6** 
	Visual Code System (VCS) **66236**				
T73	**5** 	1 	**2** 	**1** 	**0** 
	Visual Code System (VCS) **51210**				
T90	**4** 	**0** 	**0** 	**1** 	**1** 
	Visual Code System (VCS) **40011**				

3.3.4 Tooth imaging

Teeth were bench dried for 10 minutes prior to imaging. White light digital images were taken (Nikon D 200 Digital camera - SLR - 10.2 Megapixel) for each tooth from each surface (occlusal, buccal, lingual, mesial and distal); The images of each tooth surface were printed and served as reference orientation images for the subsequent images taken with other cameras. QLF images were taken for each tooth from all the surfaces under American Standards Association (ASA) Class 1 darkroom conditions using the laboratory jack platform on top of which the QLF camera was held in a fixed location. Fine focus was achieved by means of the jack platform vertical control (Figure 3.3). QLF is based on fluorescence where blue light peak intensity of 405 nm illuminates and excites tooth tissue. A low cut-off filter 520 nm is used in front of an intraoral CCD camera lens to exclude the excitation beam from the image made by the camera (Figure 3.4).

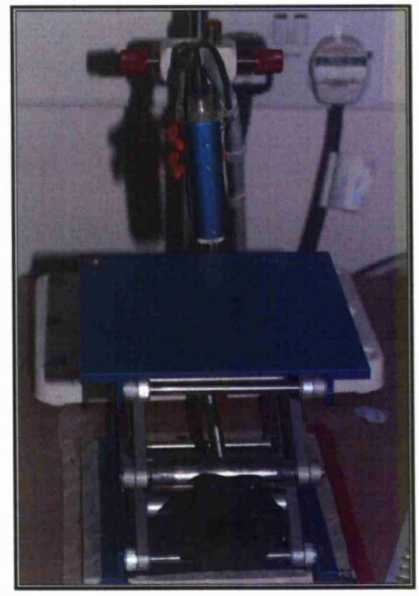


Figure 3.3: Laboratory jack platform with QLF camera on its top.

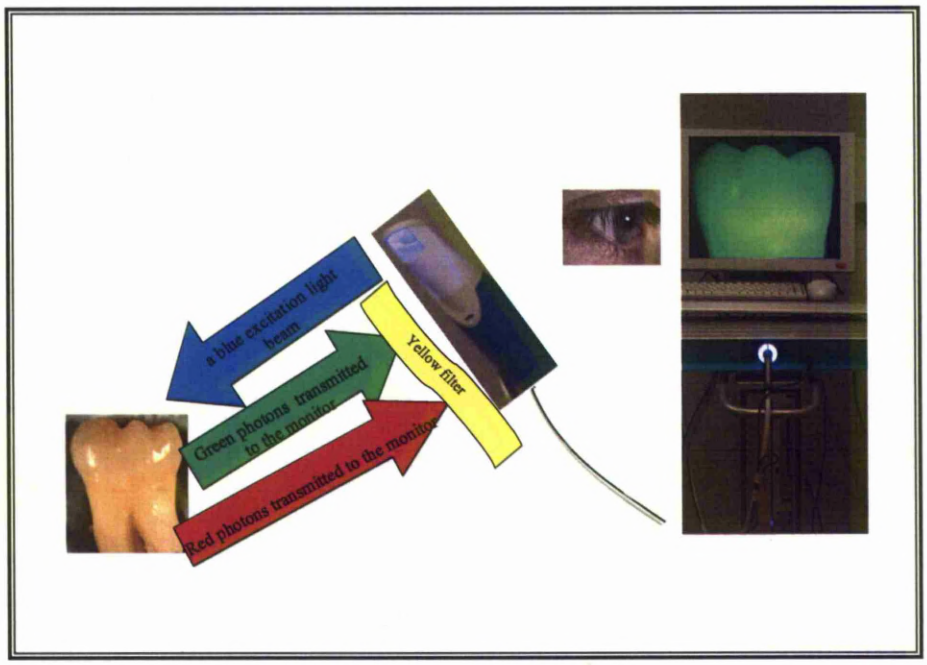
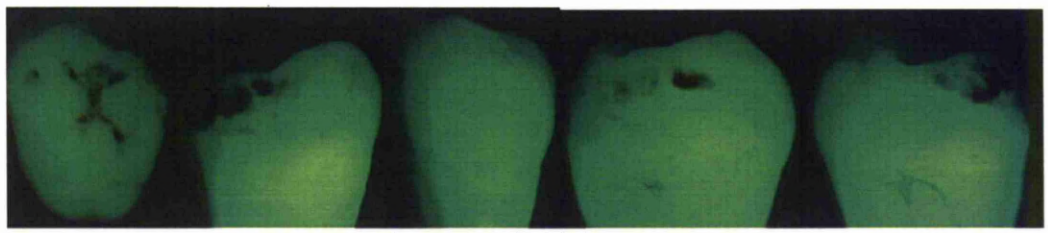


Figure 3.4: QLF concept.

Using this technique, high quality images were obtained. Finally Morita Camera images for each tooth were captured in the same way described above. A total of 1500 images were taken for this part of the study. An example of images taken with white light, QLF and the Morita cameras are shown in Figure 3.5.

Example of White-light digital images.



Example of QLF images.

Example of Morita Camera images.

Figure 3.5: Images taken for each tooth surface.

3.3.5 Radiographs

Periapical radiographs (Kodak Insight Dental Film, New York, USA) were taken for each tooth (n=100) in a standard X-ray unit (The Kingsway, Dental X-ray outfit, Watsons & son, England) (Figure 3.6). Each tooth was stabilised on the film by its root using Blu-Tack® (Bostik, England). Exposure time was varied according to tooth type (0.6 seconds for premolars and 0.7 seconds for molars) and each film processed in the normal manual way in a dark room. For each group of X-ray films made, fresh solutions prepared followed the manufacturer's instructions. Each film was developed and dried as shown in Figure 3.7 and Figure

3.8. A radiographic index (Ekstrand et al., 1997) with a five-point ranked scoring system was used (Table 3.3).

Figure 3.6: Dental X-ray unit.

Figure 3.7: Preparation for developing the films.

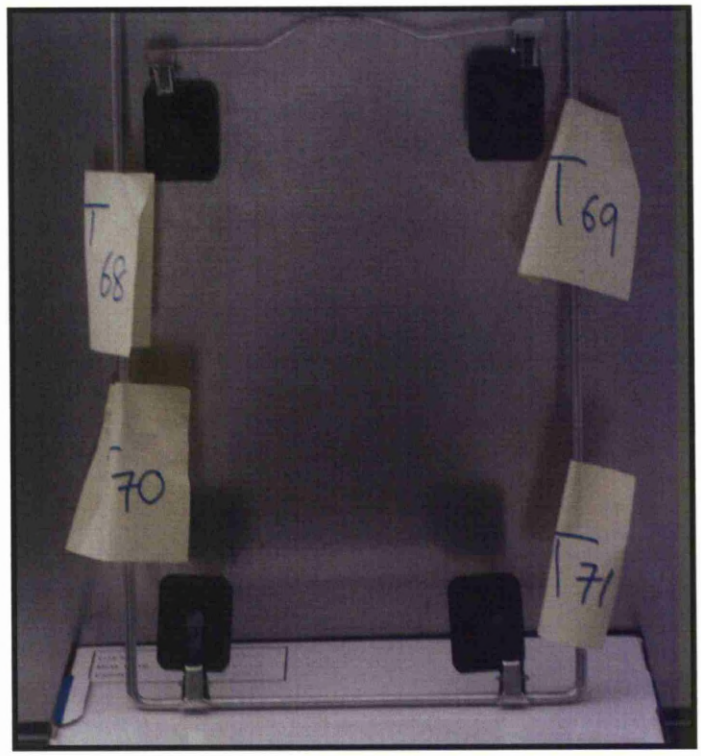


Figure 3.8: Films developed and dried at room temprature.

Table 3.3: Criteria used in the radiographic examination.

Score	Description
R0	No visible radiolucency.
R1	Radiolucency visible in the enamel.
R2	Radiolucency visible in the dentine but restricted to the outer third of the dentine.
R3	Radiolucency extending to the middle third of dentine.
R4	Radiolucency in the pulpal third of dentine.

Each film was examined and categorised following the same examiner procedure as in visual index. A value was assigned and the process was repeated twice. Finally an image of each radiograph (while it was on the X-ray viewer box) was taken (example shown in Figure 3.9).

Figure 3.9: Example of periapical radiograph.

3.3.6 Histology

Comparison of QLF measurements with histology and Transverse Microradiography (TMR) were made. Initially, the root component was removed horizontally at the enamel cementum junction (ECJ) using a handpiece with a diamond disc (Skillbond, UK). The roots were discarded and the crown portion retained for further investigation. Subsequently each crown was sectioned using a diamond wire saw (Well Wire Saw, The Precision Diamond Wire Saw Series 3, Switzerland) (Figure 3.10) from mesial to distal through the crown of the tooth into two halves. Each half was examined with a stereomicroscope (SMZ 10, Nikon) (Figure 3.11) and scored using a five-point ranked histological scoring system developed by Ekstrand, Ricketts and Kidd (ERK) by which the depth of the lesion was assessed (Ekstrand et al., 1997) (Table 3.4). An image of the half of the tooth indicating deeper carious involvement was then taken using a digital microscopy camera (Moticam 2300, 3.0 M Pixel, China). Again, each section was scored twice.

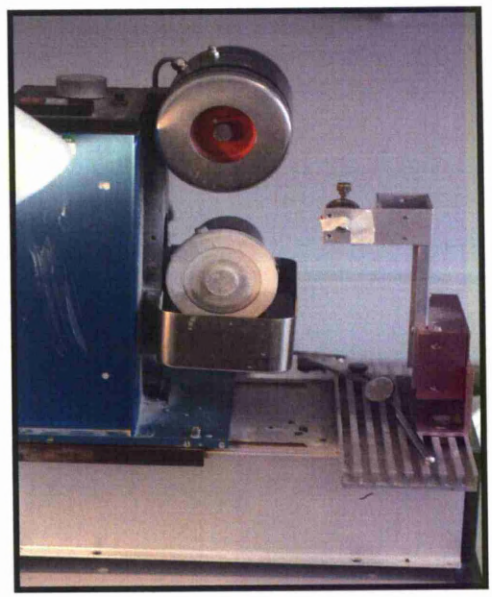


Figure 3.10: Well diamond wire saw machine.

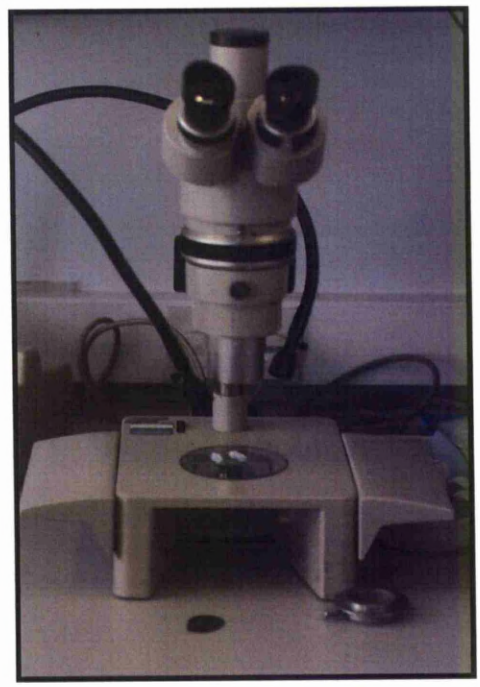


Figure 3.11: A Steromicroscope (SMZ 10, Nikon).

Table 3.4: Criteria used in the histological examination.

Score	Meaning
0	No enamel demineralisation or a narrow surface zone of opacity (edge phenomenon).
1	Enamel demineralisation (opacity) limited to the outer 50% of the enamel layer.
2	Demineralisation (brown discoloration) involving between 50% of the enamel and 1/3 of the dentine.
3	Demineralisation (brown discoloration) involving the middle third of the dentine.
4	Demineralisation (brown discoloration) involving the inner third of dentine.

3.3.7 Sections under QLF camera

The two halves produced were imaged using the QLF camera (Figure 3.12). For this part of the study a total of 200 images were obtained.

Figure 3.12: Example of two halves of a tooth under QLF.

3.3.8 TMR

Variable numbers of sections were produced using a diamond wire saw (Well Wire Saw, The Precision Diamond Wire Saw Series 3, Switzerland) in the range of 120 to around 200 microns. Each half was sectioned in a mesial to distal orientation. Sections were polished using a diamond grinding disc and custom brass anvils

(Figure 3.13) on both sides to ensure that the sections were planoparallel with an end thickness of 80 microns.

Figure 3.13: A diamond grinding disc and an anvil with tooth sections.

To confirm the mineral loss, the teeth were analysed using TMR. Each section was mounted on to a microradiographic plate-holder with an aluminium step wedge (25 μm) on glass emulsion plates (Kodak type 1A). The sections' code number was placed on the plate from the back opposite to the section. Then, plates were exposed to Cu Kα X-rays at 20 Kv and 30 mA at a distance of 30 cm for 35 minutes. The glass plates were developed in a conventional way using Kodak branded materials following manufacturers' instructions. All the plates were then rinsed in coldwater for 15 minutes and air dried. A total of 10 teeth were prepared using this technique to evaluate the appropriateness of this technique in this study

3.3.9 Micro CT

The teeth were scanned using a Micro CT 40 from Scanco (SCANCO, USA), which allowed a spatial resolution of up to 6 μm. Each tooth was inserted and stabilised inside the x-ray specimen tube using foam sponges to stop movement during scanning. The Micro CT system consisted of a combination of an X-ray shadow microscope system (Microfocus X-ray tube with a high-voltage power supply and a specimen stage with a precision manipulator) and a two-dimensional X-ray CCD camera connected to a frame-grabber and a Dual Pentium computer with colour monitor and tomographic reconstruction software. X-ray source tension/current in the range of 55 KVp/144 μA was used. Two-dimensional tomographic images were obtained from each tooth (Figure 3.14), the number depending on the size of the sample. Using the density-measuring program in the two-dimensional analysing software and following the direction in the user's guide, the mineral concentration within the demineralised tissue and adjacent immediate sound tissue were respectively measured from tomographic images to calculate the linear attenuation coefficient, mu (cm^{-1}) for determination of the change in mineral concentration of the tissue following demineralisation.

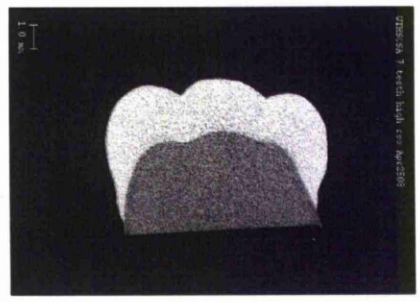


Figure 3.14: Example of an image produced by Micro CT.

3.4 Analysis

3.4.1 QLF analysis

All the QLF images were stored on the QLF PC. Each image was analysed by a single examiner for white spot (WS) and red fluorescence (RF). Analysis of the tooth surfaces was conducted by a single, blinded examiner using QLF software Inspektor Pro 2.0.0.39 (Inspektor Research System BV, Amsterdam, The Netherlands). For each tooth surface there was an image captured and stored on the QLF PC. The lesion was marked on the screen to ensure analysis of the areas determined as caries. For every lesion, the ΔF (%), the area of the lesion (mm^2) and ΔQ (the product of these two parameters, %. mm^2) were calculated by the software (Inspektor Pro 2.0.0.39, Inspektor Research System BV, Amsterdam, The Netherlands). For green fluorescence loss, a border was drawn around the lesion and from this the sound tissue fluorescence values were determined. The comparison of sound tissue fluorescence levels with the actual fluorescence values at a threshold of 5% level. The analysis resulted in a pseudo-colour image of the lesion with the different colours representing distinct levels of fluorescence loss. The value for maximum fluorescence loss were determined manually by moving the cursor over the lesion analysis image at the area of highest fluorescence loss and determining the actual value of fluorescence loss in that area; this was performed only for lesions on the occlusal surface. The average fluorescence loss was automatically calculated by the software.

RF analysis was conducted in the same way for each lesion, with the sound tissue value determined in a reference area, after which the lesion area required for analysis was selected. Using the red fluorescence levels of healthy tissue, the average level of red fluorescence in the lesion was determined at a threshold of 20%. ΔR (%) and red fluorescence area (mm^2) calculated by the software.

The sound fluorescence radiance values inside the patch were reconstructed through two-dimensional linear interpolation of sound enamel values on the patch borders. By using the pixel values of the sound enamel to rebuild the surface of the tooth and then subtracting the pixel values which were considered as a lesion. Thus, the decrease in fluorescence was determined by calculating the percentage difference between real and reconstructed fluorescence surface (De Josselin de Jong et al., 1995). The ΔQ, ΔF and WS area together with ΔR and RF areas values were recorded. After completion of the QLF analysis of a total of 1000 images produced for both WS and RF for each surface, the data were decoded and entered into SPSS for statistical analysis.

For the sections produced during histological examination which were imaged using QLF; each section was analysed for White spot and red fluorescence. For this part of the study 400 images were produced after analysis (Figure 3.15).

Figure 3.15: Example of histological sections analysis by QLF.

3.4.2 Morita-Penviewer images analysis

All the Morita images were stored on the QLF PC. A total of 500 images were analysed for red fluorescence using the QLF software. ΔR (%) and RF area (mm^2) values were recorded for each tooth surface. The data were decoded and entered into SPSS for statistical analysis.

3.4.3 TMR analysis

The microradiograph plates were examined with a Leica microscope (Leica, Brighton, England) with images captured by means of a CCD video camera (Sony, Tokyo, Japan) linked to a computer (Viglen PC, UK) at a magnification of 20x/ 0.40. The sections were analysed by means of a software package (TMRW v. 1.22, Inspektor Research System BV, Amsterdam, The Netherlands).

All data gathered from different indices, systems and techniques employed in this study and exported into word files for each tooth (Example- Appendix 1).

3.5 Statistical Methods

The data were statistically analysed using SPSS for Windows software (version 17.0). After the descriptive analysis of the data, ΔF, ΔQ, ΔR for QLF and ΔR for Morita, they were compared with ICDAS II for the whole values and for the mean of each parameter for each tooth surface by the use of box plots. Quantitative data were described by means, median and standard deviation.

Spearman's correlation coefficient was employed to compare QLF values, ICDAS II scores and histology scores on the occlusal surface. On all the surfaces of the teeth, for each ICDAS II score the confidence intervals for green fluorescence loss and red fluorescence level was determined and translated into a QLF average green fluorescence loss and red fluorescence level index. Specificity and sensitivity were calculated as well as the quality of the intra-examiner reproducibility using a kappa statistic.

3.6 Results

3.6.1 Occlusal surface

3.6.1.1 ICDAS II with ΔF

The results presented in Figure 3.16 show that ΔF at the 5% threshold level correlated positively with ICDAS II visual index. It can be clearly seen that as the ICDAS II increased the average green fluorescence loss increased, therefore, as the severity of dental caries increases, the fluorescence loss increases.

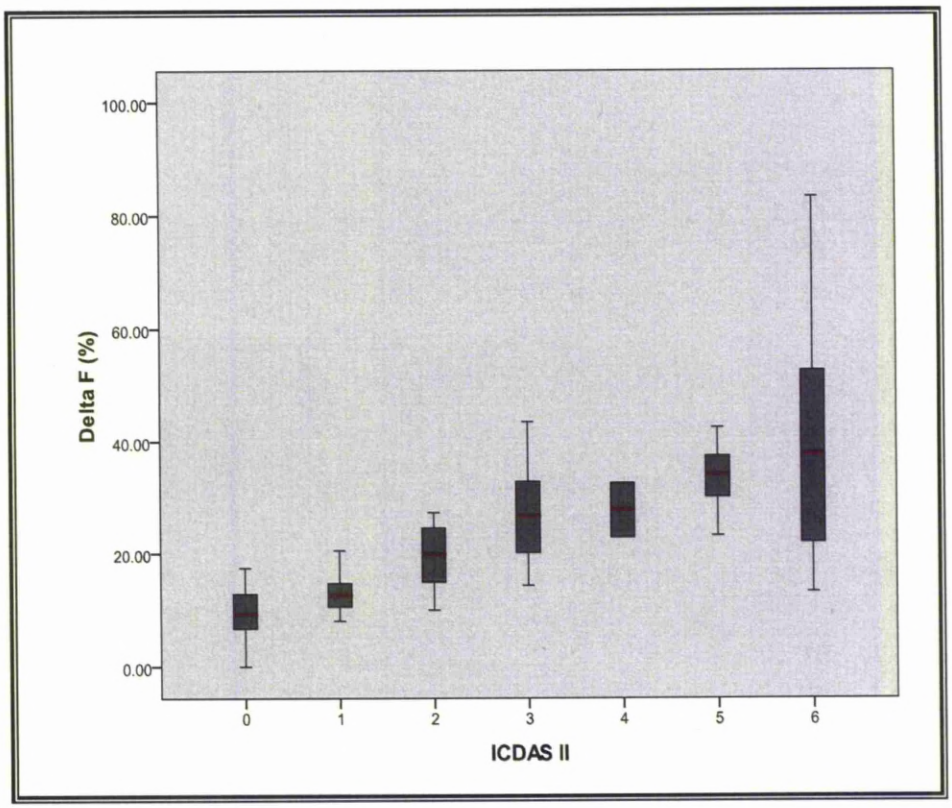


Figure 3.16: Graph showing ICDAS II scores against ΔF (%) on occlusal surfaces.

The data presented in Figure 3.17 show a positive relation between ICDAS II and maximum fluorescence loss which correlated more with ICDAS II (0.795) than average ΔF (0.772). This was significant at the 1% level.

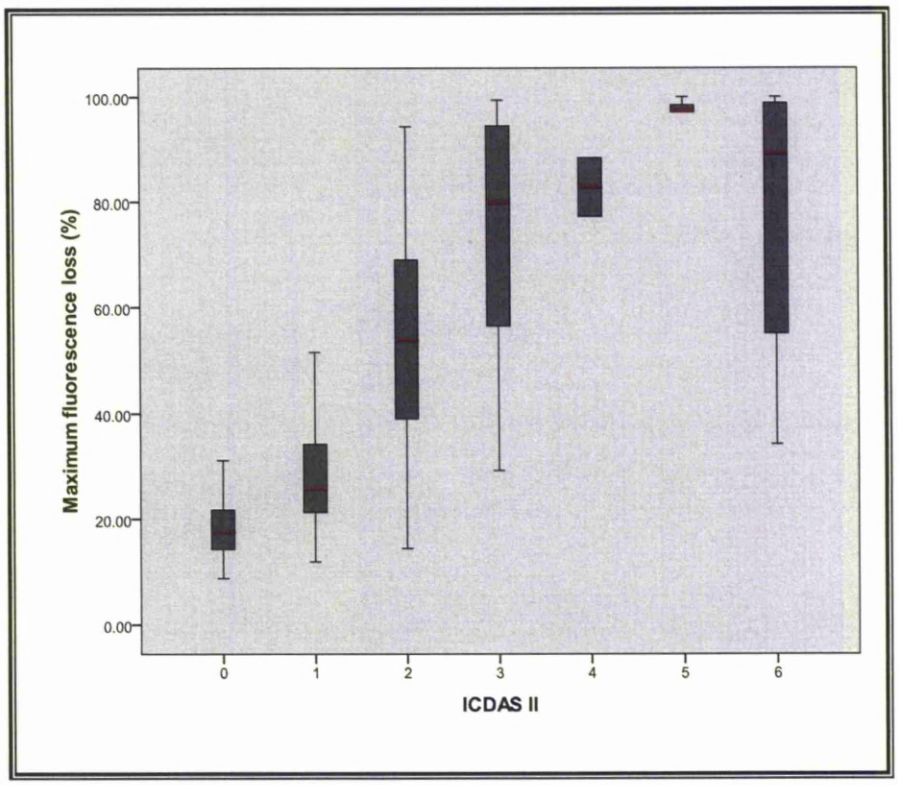


Figure 3.17: Graph showing ICDAS II scores against values for maximum fluorescence loss (%) on occlusal surfaces.

3.6.1.2 ICDAS II with ΔQ

Figure 3.18 shows that ΔQ (area×ΔF) correlated positively with ICDAS II visual index, with a correlation of 0.710, which was significant at the 1% level.

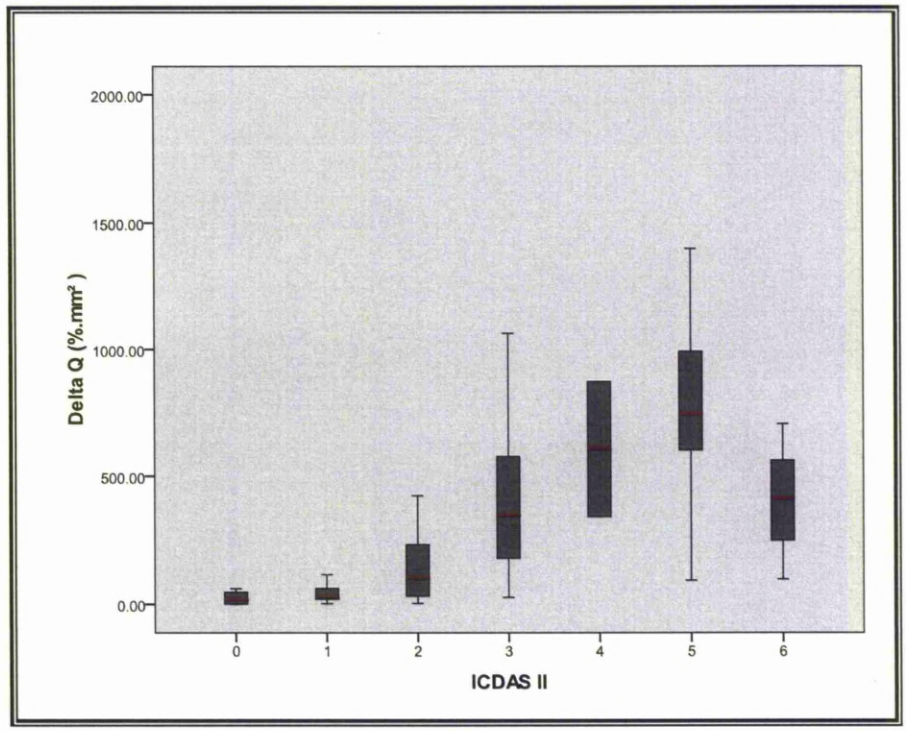


Figure 3.18: Graph showing ICDAS II scores against values for ΔQ (%.mm^2) on occlusal surfaces.

3.6.1.3 ICDAS II with ΔR-QLF and ΔR Morita

The results presented in Figure 3.19 show that red fluorescence level ΔR-QLF correlated positively with the ICDAS II visual index especially in its early stages (ICDAS II scores 0, 1, 2 and 3) as well as ΔR-Morita which correlated positively with the ICDAS II visual index. In comparison with images taken by the Morita camera, this camera system detected more red fluorescence than QLF.

Morita camera images gave better correlation with ICDAS II than QLF. The increase in ΔR-Morita values were more strongly correlated to the increase in ICDAS II scores (0.738) than the ΔR-QLF values (0.634).

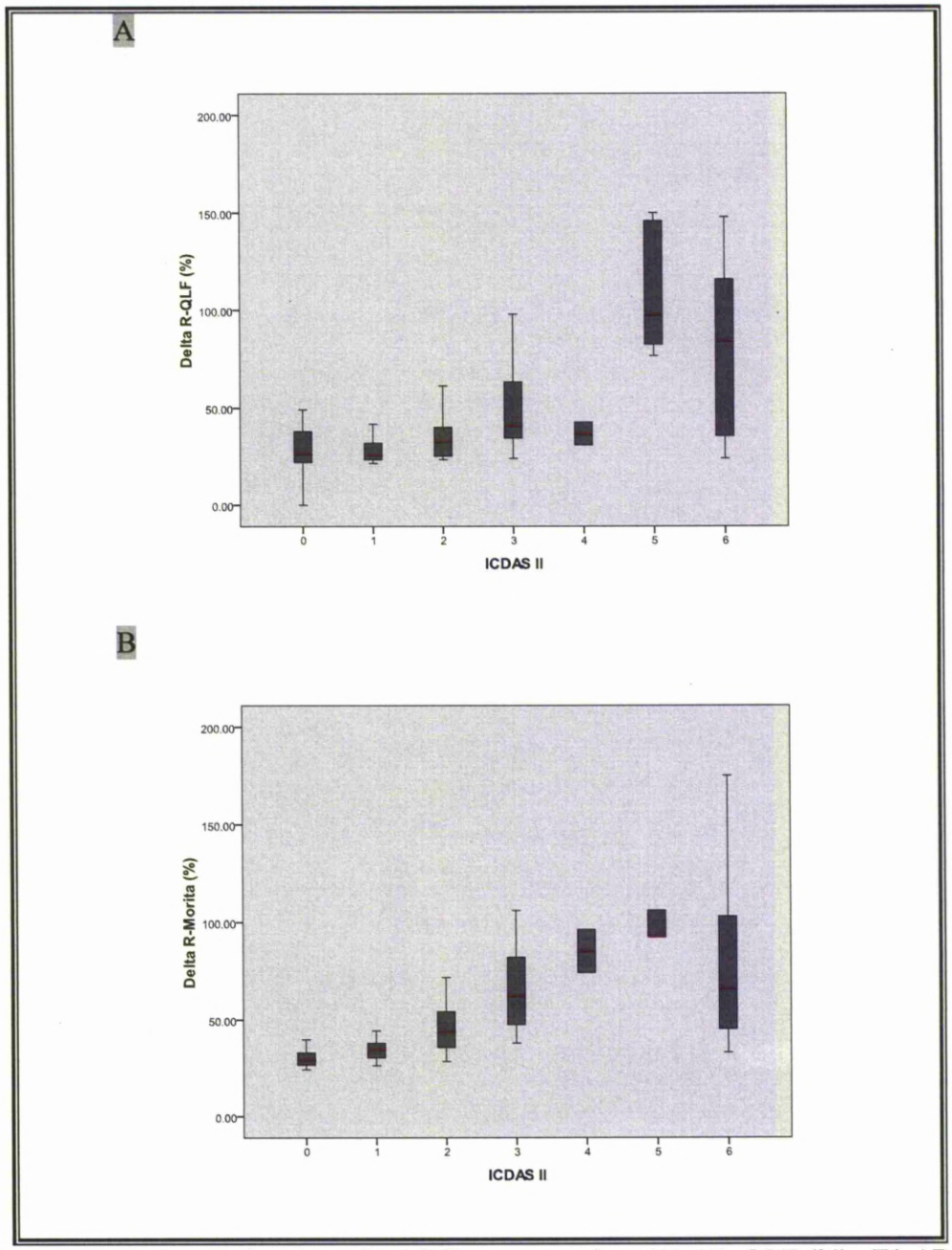


Figure 3.19: Graph showing ICDAS II scores against (A) ΔR-QLF (%), (B) ΔR-Morita (%) for occlusal surfaces.

3.6.1.4 Histology with ΔF

ΔF QLF parameter in this study correlated positively with histology (Figure 3.20).

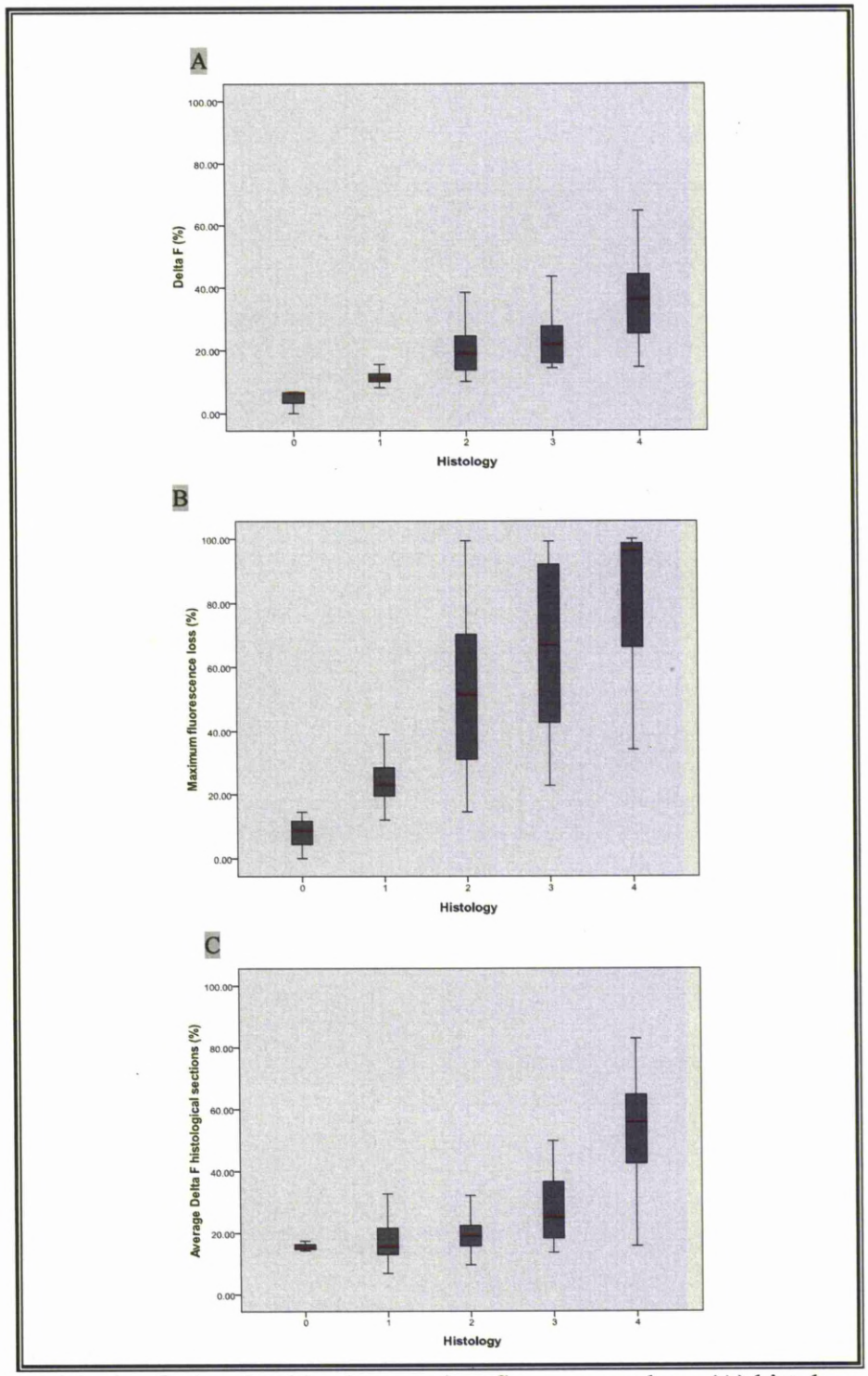


Figure 3.20: Graph showing histology against fluorescence loss, (A) histology with ΔF (%) on occlusal surfaces, (B) histology with maximum fluorescence loss (%) on occlusal surfaces and (C) histology with ΔF (%) sections under QLF conditions.

Figure 3.20-A shows positive correlation of 0.753 between histology (gold standard) and ΔF values obtained from the analysis of the occlusal surfaces of the teeth, correlation was significant at the 1% level. Figure 3.20-B shows the correlation of 0.696 between histology and maximum fluorescence loss on the occlusal surfaces of the teeth, which was significant at the 1% level. The correlation between histology and the average fluorescence loss of teeth's sections was positive (0.601) as shown in Figure 3.21-C. Figure 3.21 shows tooth sections under QLF conditions. Figure 3.22 shows the amount of demineralisation extension into dental tissues and some red fluorescence.

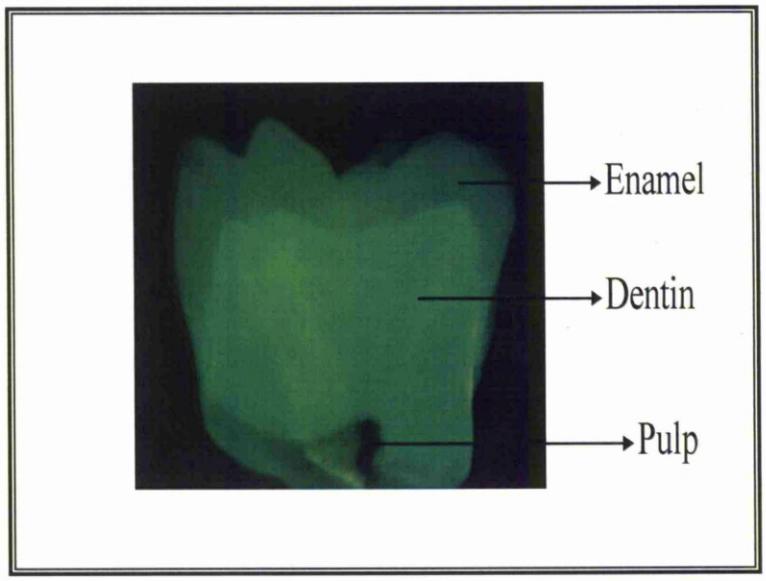


Figure 3.21: The histological section under QLF camera.

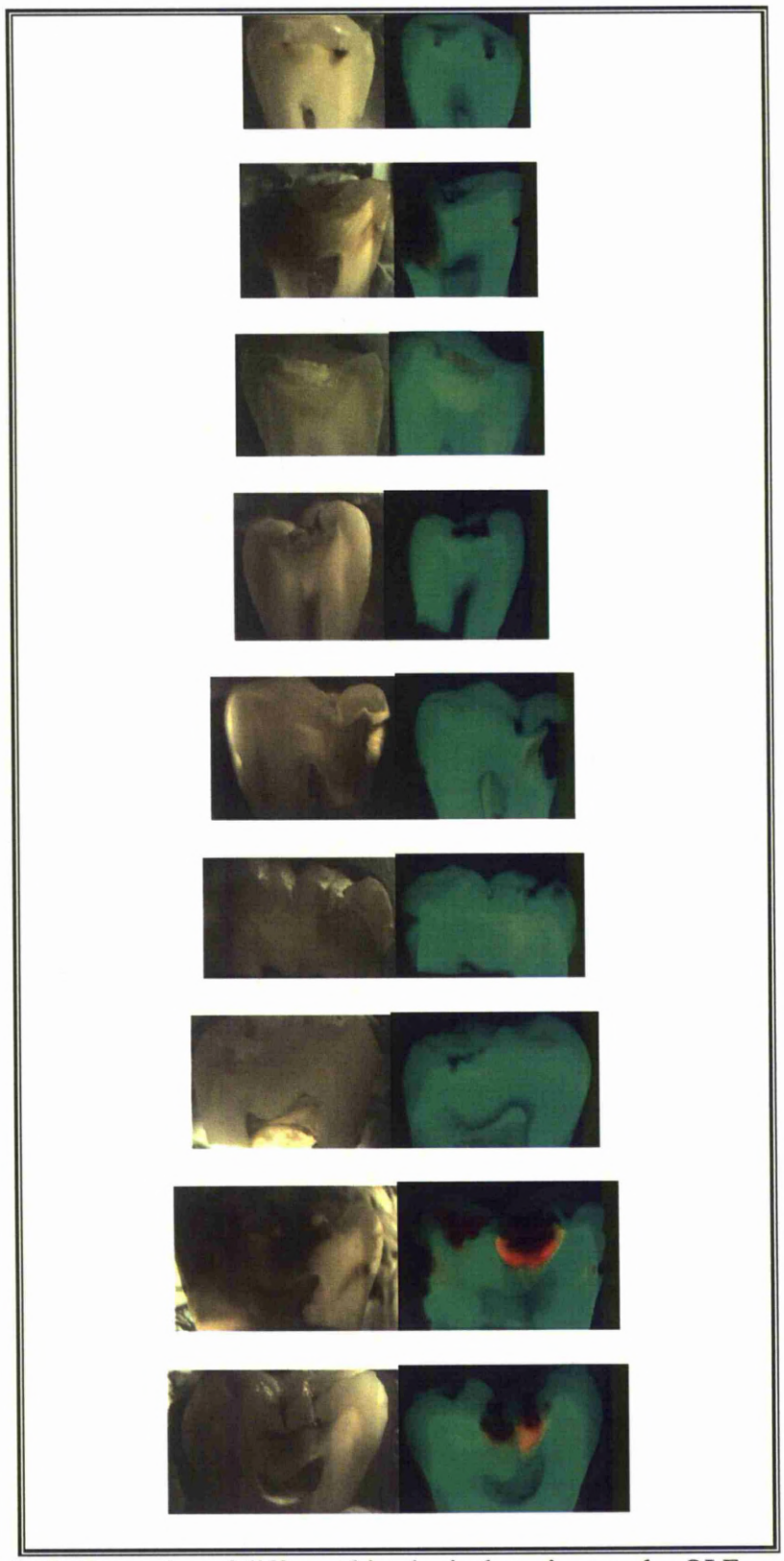


Figure 3.22: Examples of different histological sections under QLF conditions showing the amount of demineralisation extension into the dental tissues and red fluorescence.

3.6.1.5 ICDAS II with histology

On the occlusal surfaces the correlation in which histological depths corresponded to the visual scores ICDAS II was significant at the 1% level (0.800). Sensitivity and specificity of ICDAS II scores with each score of histological index is presented in Table 3.5.

Table 3.5: Sensitivity and specificity of ICDAS II with histology.

Histology Score	Sensitivity	Specificity
0	1	1
1	0.82	0.85
2	0.91	0.72
3	0.82	0.86
4	0.83	0.74

3.6.1.6 Radiograph

On the occlusal surfaces radiographs correlated less perfectly with ICDAS II (0.670), ΔF (0.601), ΔR-QLF (0.412), ΔR-Morita (0.503) and histology (0.591).

3.6.1.7 Micro CT

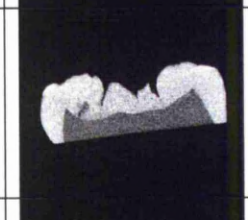
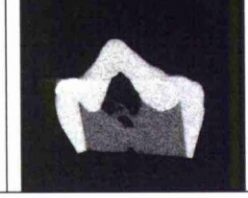
The percent change in mineral density was calculated as follows:

% change in Linear Attenuation Coefficient (LAC) (cm^{-1}) =

$$\frac{\text{LAC (Sound area)} - \text{LAC (demineralised area)}}{\text{LAC (sound)}} \times 100$$

Results obtained using μCT is presented in Figure 3.23. It was observed that as dental caries increased in severity the percentage change in mineral density increased as shown in Table 3.6.

Table 3.6: Example of results obtained from Micro CT.

Sample code	Demineralised (A)		Sound (B)		Image
	Enamel	Dentine	Enamel	Dentine	
T23	2.3	3.89	8.00	4.42	
T37	1.93	4.89	8.00	6.28	
T90	6.74	1.17	8.00	5.97	

Values of the Linear Attenuation Coefficient (cm^{-1}).

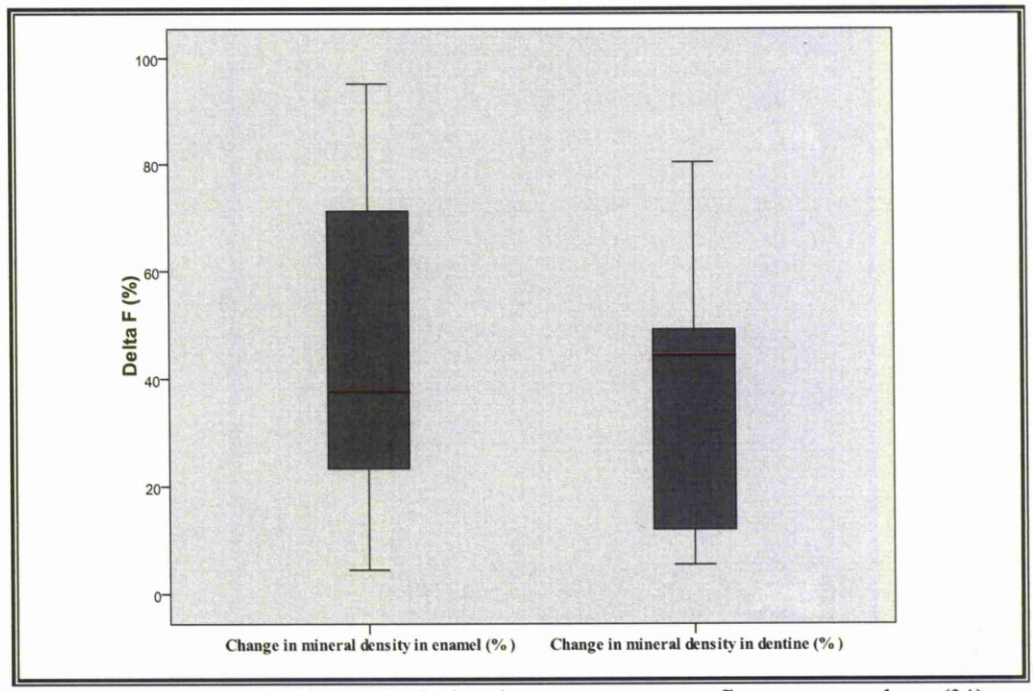


Figure 3.23: Graph showing correlation between average fluorescence loss (%) and percent change in mineral density in enamel and dentine.

3.6.1.8 The Transverse Microradiography (TMR)

The method has been used for the confirmation of the presence of demineralisation. Figure 3.24 shows surface softening and subsurface lesion.

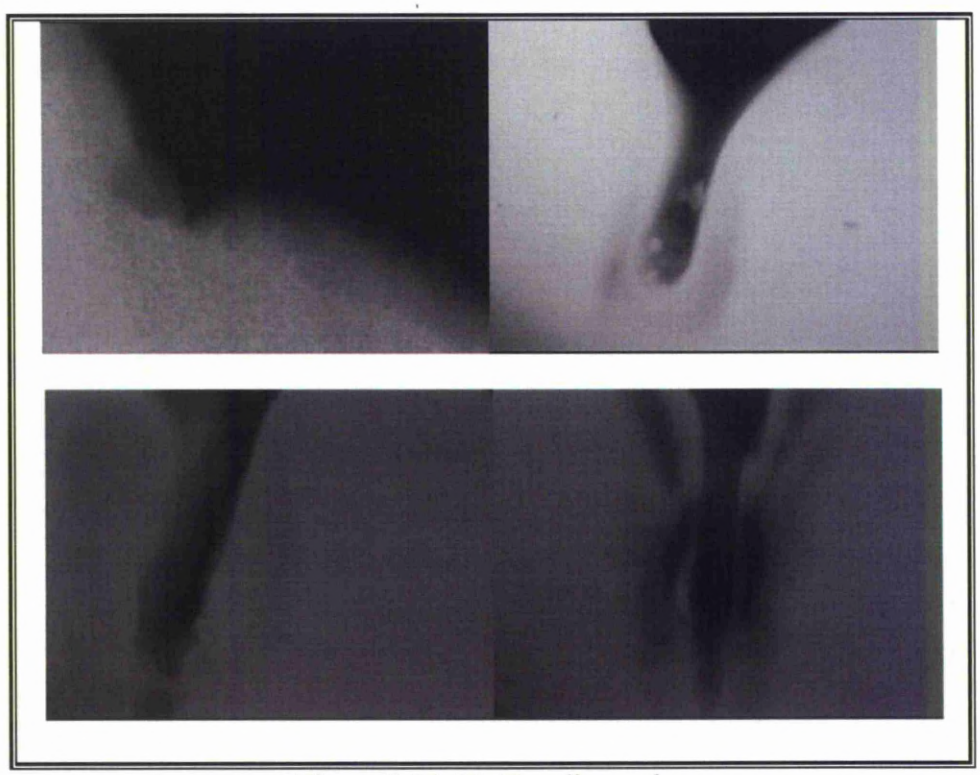


Figure 3.24: TMR radiographs.

3.6.1.9 QLF occlusal surface index and their corresponding histological and ICDAS II classifications

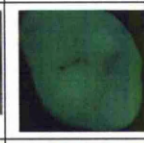
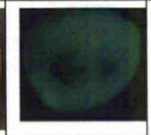
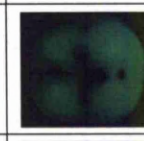
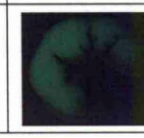
The researcher's point of view regarding the selection of a 5 points index score for QLF in this study was to make it easier for clinicians to link QLF findings with histological extension, which is the most important factor in decision making for treatment action as shown in Table 3.7 which was gathered and developed by the researcher in this study.

Table 3.7: The classification of QLF index according to histology and ICDAS II scores.

QLF Score	Description/ Action	Histology Score	Description	ICDAS II	Description
QLF 0	No dark spots/ no treatment.	**0**	No enamel demineralisation.	**0**	Sound
QLF 1	First visual change in green fluorescence/ preventive treatment and monitoring.	**1**	Enamel demineralisation limited to its outer 50%.	**1**	Brown/white first visual change in enamel.
QLF 2	Distinct Visual change in green fluorescence and or appearance of orange red fluorescence/ preventive and or simple conservative restorative treatment.	**2**	Demineralisation between 50% of the enamel and 1/3 of dentine.	**2**	Brown/white distinct visual change in enamel.
QLF 3	Distinct dark spots and appearance of orange red fluorescence/ classic restorative treatment.	**3**	Demineralisation involving the middle 1/3 of the dentine.	**3& 4**	Localised enamel break-down and /or underlying shadow.
QLF 4	Extensive fluorescence loss and dark spots with orange red fluorescence/ Invasive restorative treatment.	**4**	Demineralisation involving the inner 1/3 of the dentine.	**5& 6**	Distinct and/ or Extensive cavity.

A preliminary index was prepared by analysing all the results and QLF scores developed with relation to histology and ICDAS II scores as in Table 3.8. The mean values and confidence intervals for each QLF parameter and corresponding ICDAS II scores were calculated for QLF parameters on occlusal surfaces and index derived. In addition, for the occlusal surface the maximum fluorescence loss and histology scores presented.

Table 3.8: Overview of QLF occlusal index and their corresponding histological and ICDAS II classifications.

Histological Classification			ICDAS II Classification			QLF Classification system			
							ΔF (%)	Max. FL (%)	ΔR (%)
C	Desc.	Example	C	Desc.	Example	Example	Index	Index	Index
0	No Enamel demin.		0	Sound.			-0.5-10	-0.5-22	0-20
1	Demin. limited to the outer ½ of enamel.		1	Brown/ white first visual change in enamel.			10.5-15	22.5-30	21-35
2	Demin. involving between inner ½ of the enamel and outer 1/3 of dentine.		2	Brown/ white distinct visual change in enamel.			15.5-25	30.5-45	36-60
3	Demin. involving the middle 1/3 of the dentine.		3	Localised enamel break-down.			25.5-30	45.5-65	61-78
			4	Under-lying shadow.			30.5-35	65.5-72	79-92
4	Demin. involving the inner 1/3 of the dentine		5	Distinct cavity.			35.5-45	72.5-85	93-99
			6	Extensive cavity.			>45.5	>85	>99

C= Code
Demin. = Demineralisation.
Des. = Description.
Max= Maximum.
FL= Fluorescence loss.

3.6.1.10 QLF occlusal index with histology

The sensitivity and specificity of QLF index with histology was positively high as presented in Table 3.9.

Table 3.9: QLF Index sensitivity and specificity with histology.

QLF Index	Histology	
	Sensitivity	Specificity
QLF 0	1	0.90
QLF 1	0.91	0.82
QLF 2	0.83	0.88
QLF 3	0.88	0.75
QLF 4	0.85	0.71

3.6.2 Buccal surface

3.6.2.1 ICDAS II with ΔF

The results obtained and presented in Figure 3.25 show that ΔF at the 5% threshold level correlated positively with ICDAS II visual index. As the ICDAS II scores increased the average green fluorescence loss increased. Therefore, as the severity of dental caries increases the fluorescence loss increases. The correlation coefficient was 0.701 and the difference was significant at the 1% level.

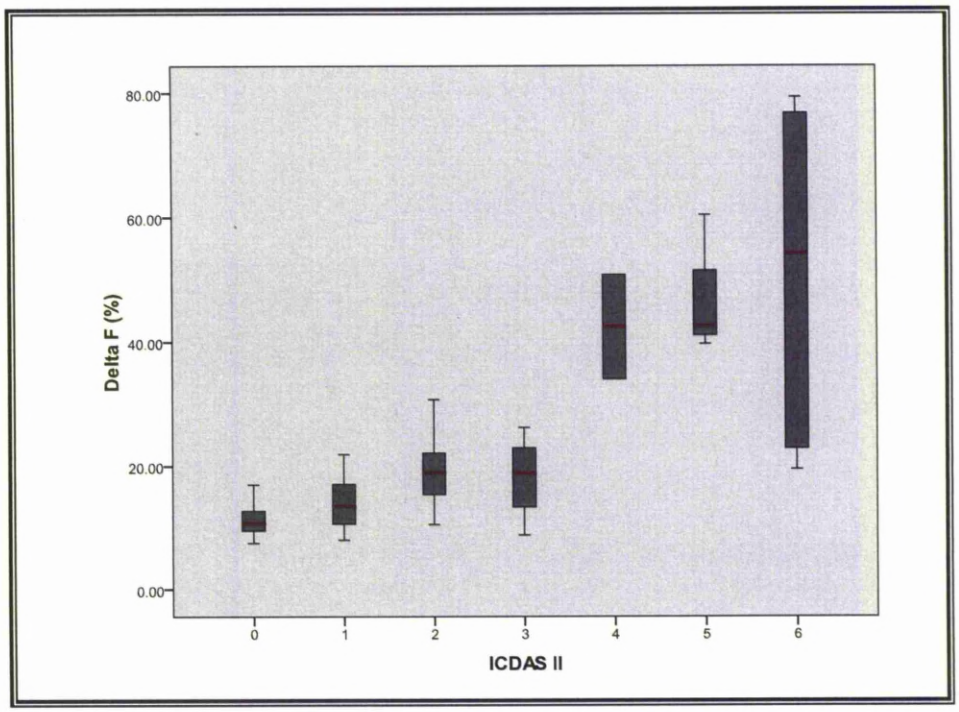


Figure 3.25: Graph showing ICDAS II scores against ΔF (%) on buccal surfaces.

3.6.2.2 ICDAS II with ΔQ

Results in Figure 3.26 show that ΔQ (area×ΔF) correlated positively with ICDAS II visual index. Correlation between ICDAS II and ΔQ was 0.733 and was significant at the 1% level.

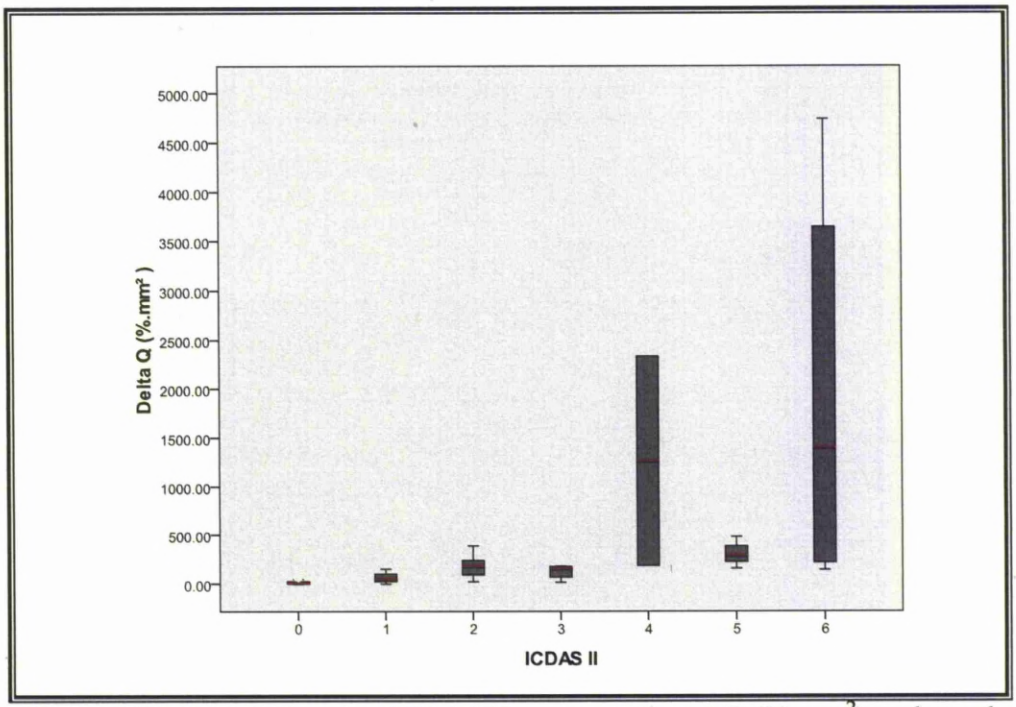


Figure 3.26: Graph showing ICDAS II scores against ΔQ (%.mm^2) on buccal surfaces.

3.6.2.3 ICDAS II with ΔR-QLF and ΔR Morita

The results presented in Figure 3.27, A and B show that the red fluorescence level ΔR-QLF correlated positively with the ICDAS II visual index especially in its early stages (ICDAS II scores 0, 1, 2, 3 and 4) as well as with ΔR-Morita. With the Morita camera, the ICDAS II scores increased linearly with the level of red fluorescence on the tooth surface and were able to detect more red fluorescence than QLF. The Morita images showed better correlation with ICDAS II than with QLF. The increase in ΔR-Morita values was more strongly correlated to the increase in ICDAS II scores (0.588) than the ΔR-QLF values (0.481).

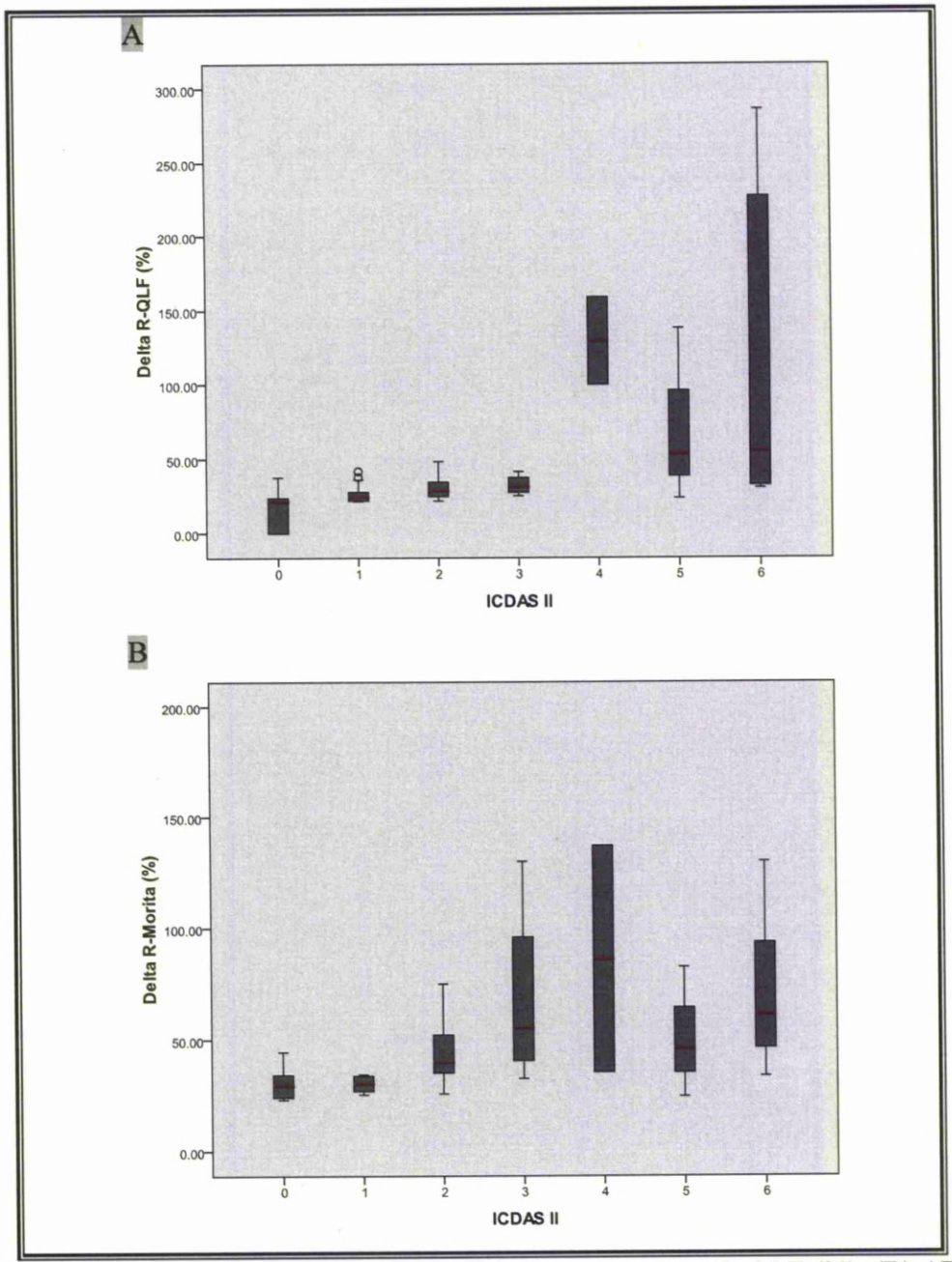
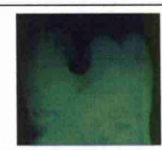


Figure 3.27: Graph showing ICDAS II scores against (A) ΔR-QLF (%), (B) ΔR-Morita (%).

3.6.2.4 QLF buccal surfaces index examples

After analysing all the data of QLF parameters on buccal surfaces, the index derived and examples are shown in Table 3.10.

Table 3.10: Examples of QLF buccal surfaces index and their corresponding ICDAS II classifications.

ICDAS II Classification			QLF Classification system			
				ΔF (%)	ΔR (%)	QLF Index Score
Code	Description	Example	Example	Index	Index	
0	Sound.			-0.5-10	0-20	**QLF 0**
1	Brown/white first visual change in enamel.			10.5-15	21-35	**QLF 1**
2	Brown/white distinct visual change in enamel.			15.5-25	36-60	**QLF 2**
3	Localised enamel break-down.			25.5-30	61-78	**QLF 3**
4	Underlying shadow.			30.5-35	79-92	
5	Distinct cavity.			35.5-45	93-99	**QLF 4**
6	Extensive cavity.			>45.5	>99	

3.6.3 Lingual surface

3.6.3.1 ICDAS II with ΔF

The results presented in Figure 3.28 show that ΔF at the 5% threshold level correlated positively with the ICDAS II visual index. As the ICDAS II increased the average green fluorescence loss increased. The correlation was 0.700 and was significant at the 1% level.

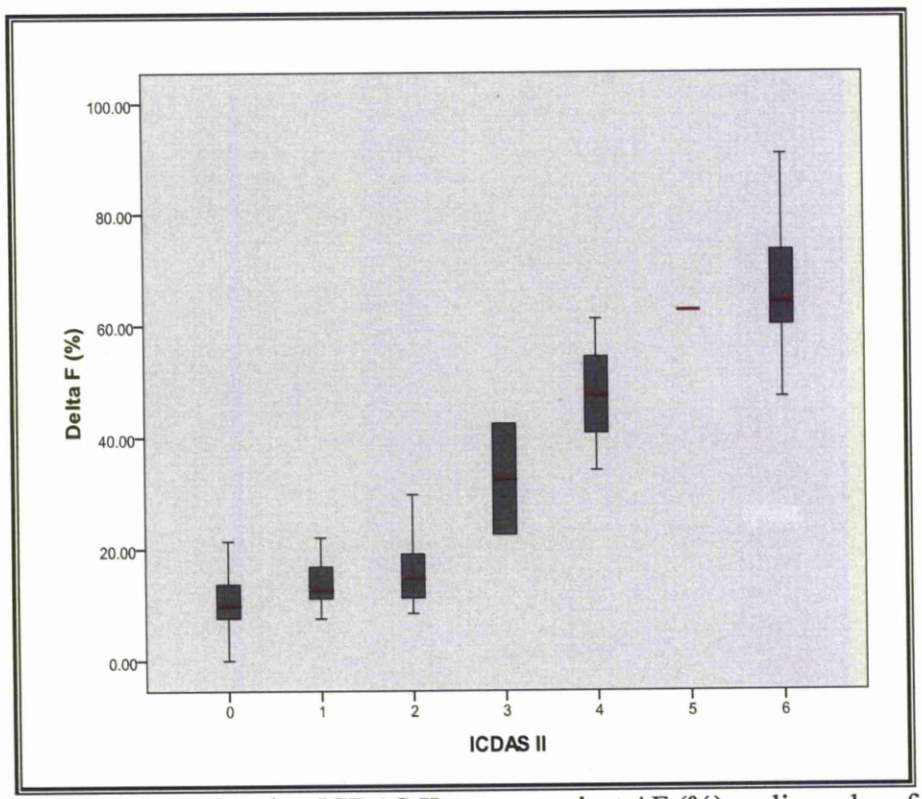


Figure 3.28: Graph showing ICDAS II scores against ΔF (%) on lingual surfaces.

3.6.3.2 ICDAS II with ΔQ

Results in Figure 3.29 show that ΔQ correlated positively with the ICDAS II visual index. Correlation between ICDAS II and ΔQ 0.716 and was significant at the 1% level.

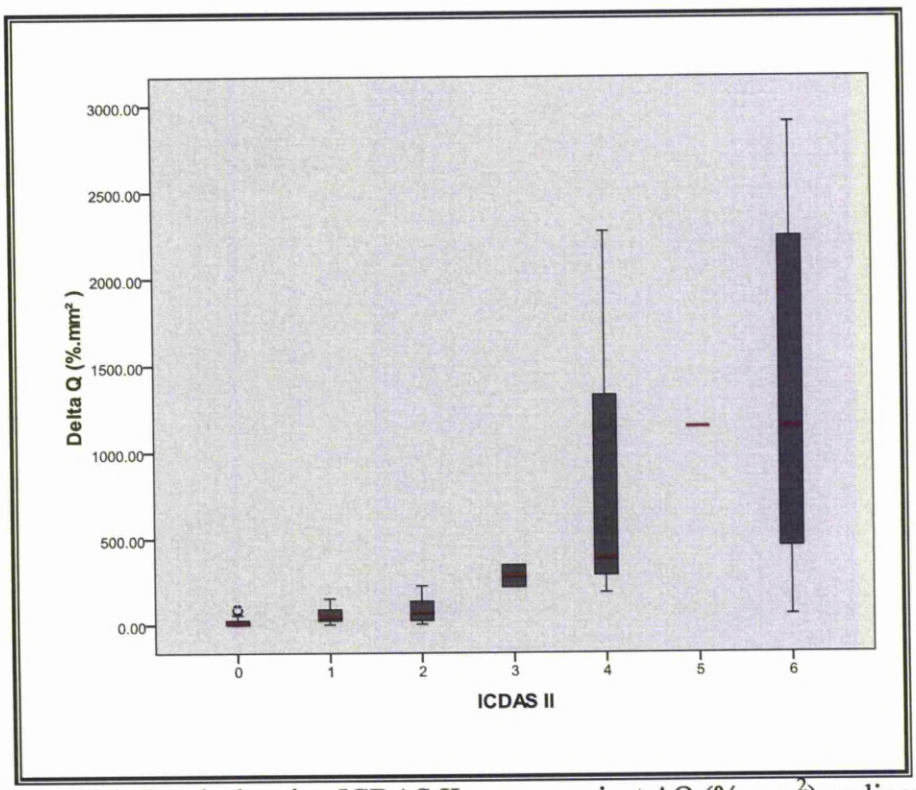


Figure 3.29: Graph showing ICDAS II scores against ΔQ (%.mm^2) on lingual surfaces.

3.6.3.3 ICDAS with ΔR-QLF and ΔR-Morita.

The results in Figure 3.30 show that there was a good correlation between ICDAS II and ΔR-QLF as well as ΔR-Morita on the lingual surfaces of the teeth in ICDAS II scores. On the lingual surfaces the correlation of ΔR-QLF with ICDAS II (0.603). It was to some extent improved than with ΔR-Morita (0.590), although this difference was not significant ($p > 0.05$).

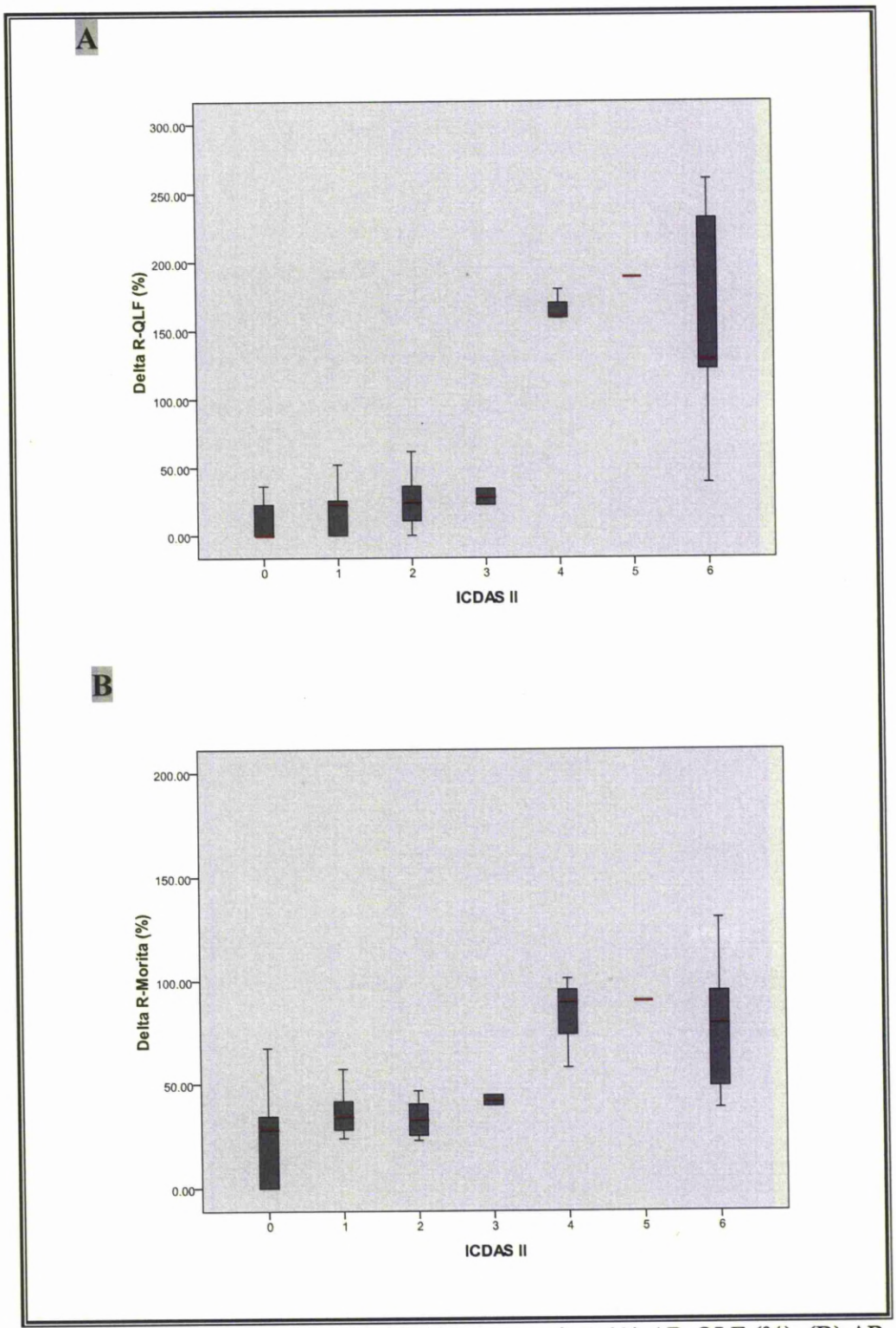
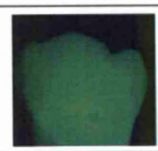
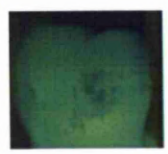
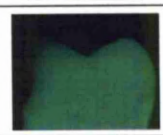


Figure 3.30: Graph showing ICDAS II scores against (A) ΔR-QLF (%), (B) ΔR-Morita (%) for lingual surfaces.

3.6.3.4 QLF lingual surfaces index examples.

After analysing all the data of QLF parameters on the lingual surfaces, the index derived and examples are shown in Table 3.11.

Table 3.11: Examples of QLF lingual surfaces index and their corresponding ICDAS II classifications.

ICDAS II Classification			QLF Classification system			
				ΔF (%)	ΔR (%)	QLF Index Score
Code	Description	Example	Example	Index	Index	
0	Sound.			-0.5-10	0-20	QLF 0
1	Brown/white first visual change in enamel.			10.5-15	21-35	QLF 1
2	Brown/white distinct visual change in enamel.			15.5-25	36-60	QLF 2
3	Localised enamel break-down.			25.5-30	61-78	QLF 3
4	Underlying shadow.			30.5-35	79-92	
5	Distinct cavity.			35.5-45	93-99	QLF 4
6	Extensive cavity.			>45.5	>99	

3.6.4 Mesial surface

3.6.4.1 ICDAS II with ΔF

The results presented in Figure 3.31 show that ΔF at the 5% threshold level correlated positively with ICDAS II visual index. As the ICDAS II scores increase the average green fluorescence loss increases, the correlation coefficient was 0.690.

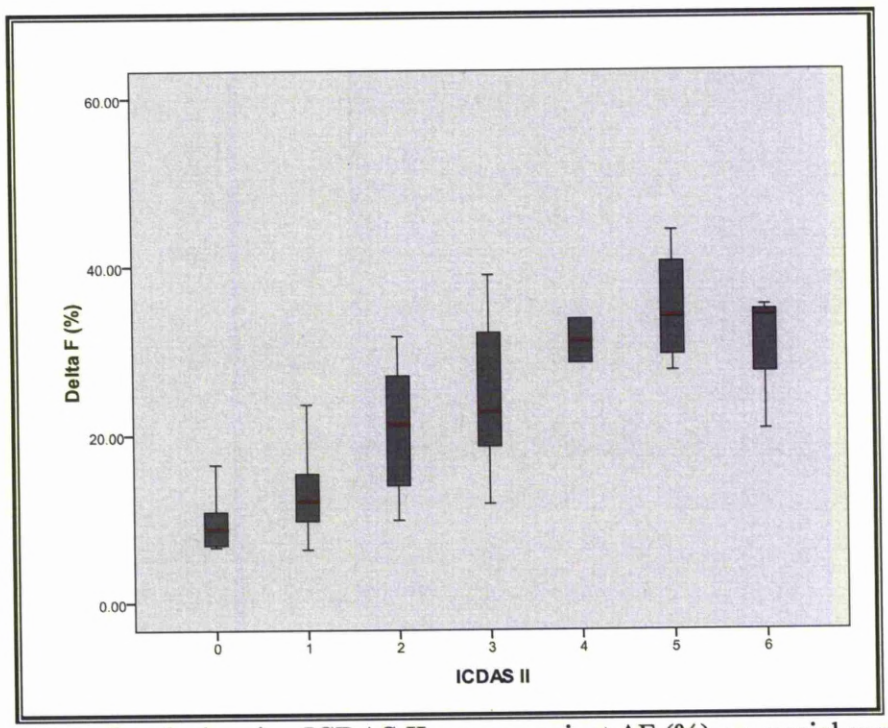


Figure 3.31: Graph showing ICDAS II scores against ΔF (%) on mesial surfaces.

3.6.4.2 ICDAS II with ΔQ

Figure 3.32 shows the ΔQ correlated positively with the ICDAS II visual index. Correlation between ICDAS II and ΔQ (0.687) was significant at the 1% level.

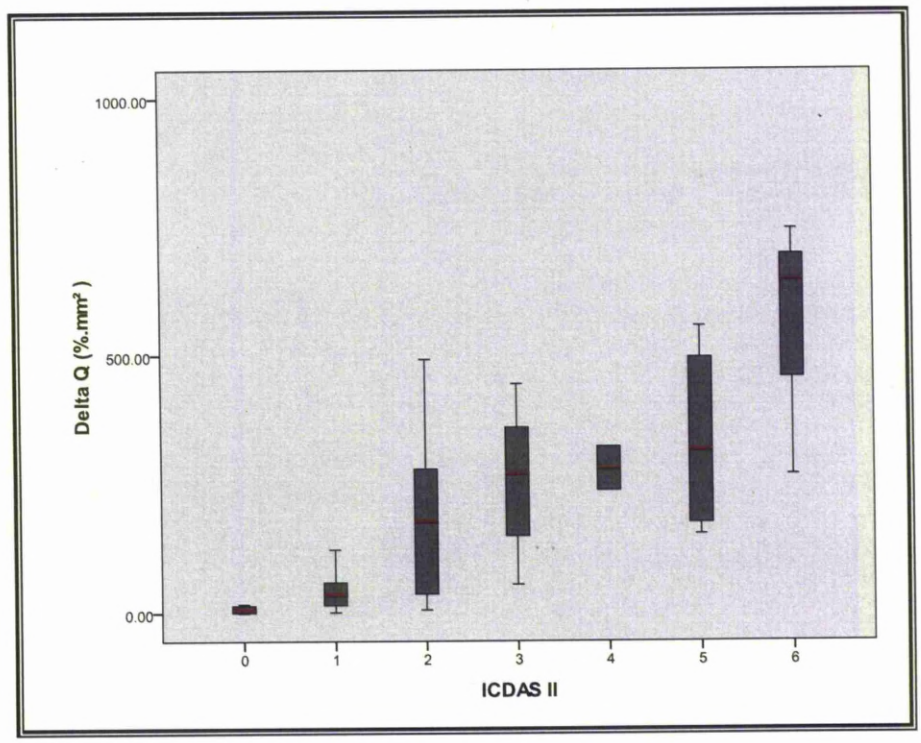


Figure 3.32: Graph showing ICDAS II scores against ΔQ (%.mm^2) on mesial surfaces.

3.6.4.3 ICDAS with ΔR-QLF and ΔR-Morita.

The results in Figure 3.33 show that there was a good correlation between ICDAS II scores and ΔR-QLF as well as ΔR-Morita on the mesial surfaces of the teeth in the early stages of ICDAS II scores. Morita camera images showed better correlation with ICDAS II than QLF. The increase in ΔR-Morita values were more strongly correlated to the increase in ICDAS scores (0.601) than the ΔR-QLF values (0.490).

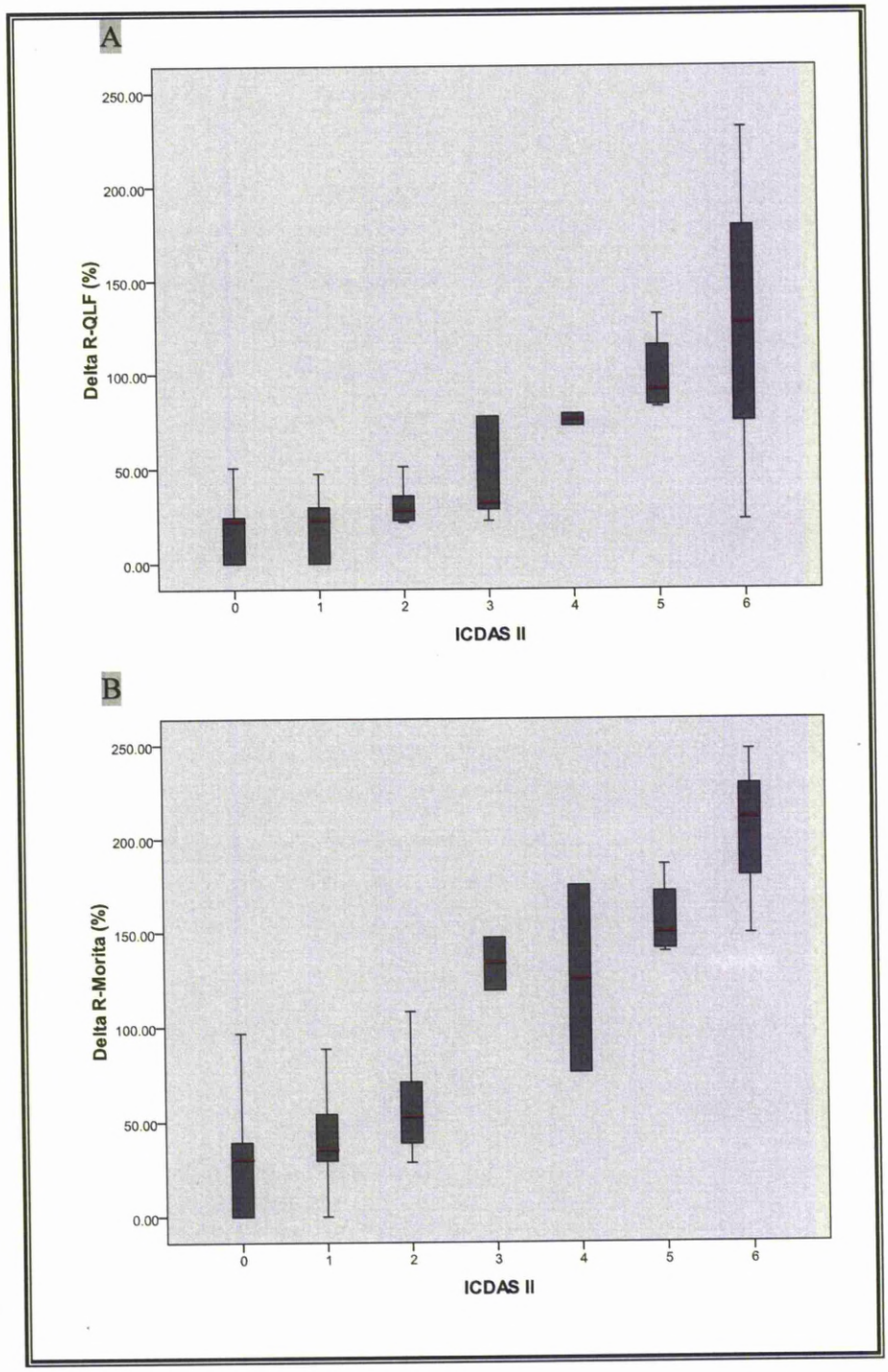
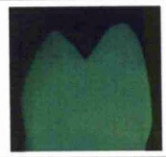
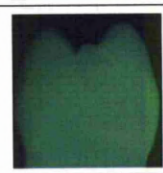
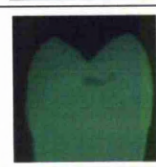
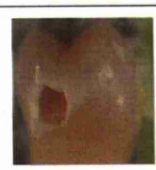
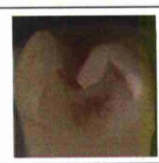
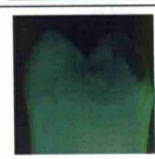


Figure 3.33: Graph showing ICDAS II scores against (A) ΔR-QLF (%), (B) ΔR-Morita (%) for mesial surfaces.

3.6.4.4 QLF mesial surfaces index examples.

After analysing all the data of QLF parameters on the mesial surfaces, the index derived and examples are shown in Table 3.12.

Table 3.12: Examples of QLF mesial surfaces index and their corresponding ICDAS II classifications.

ICDAS II Classification			QLF Classification system			
				ΔF (%)	ΔR (%)	QLF Index Score
Code	Description	Example	Example	Index	Index	
0	Sound.			-0.5-10	0-20	QLF 0
1	Brown/white first visual change in enamel.			10.5-15	21-35	QLF 1
2	Brown/white distinct visual change in enamel.			15.5-25	36-60	QLF 2
3	Localised enamel break-down.			25.5-30	61-78	QLF 3
4	Underlying shadow.			30.5-35	79-92	
5	Distinct cavity.			35.5-45	93-99	QLF 4
6	Extensive cavity.			>45.5	>99	

3.6.5 Distal surface

3.6.5.1 ICDAS II with ΔF

The results presented in Figure 3.34 show that ΔF at the 5% threshold level correlated positively with the ICDAS II visual index. As the ICDAS II scores increased the average green fluorescence loss increased with a correlation coefficient of 0.750.

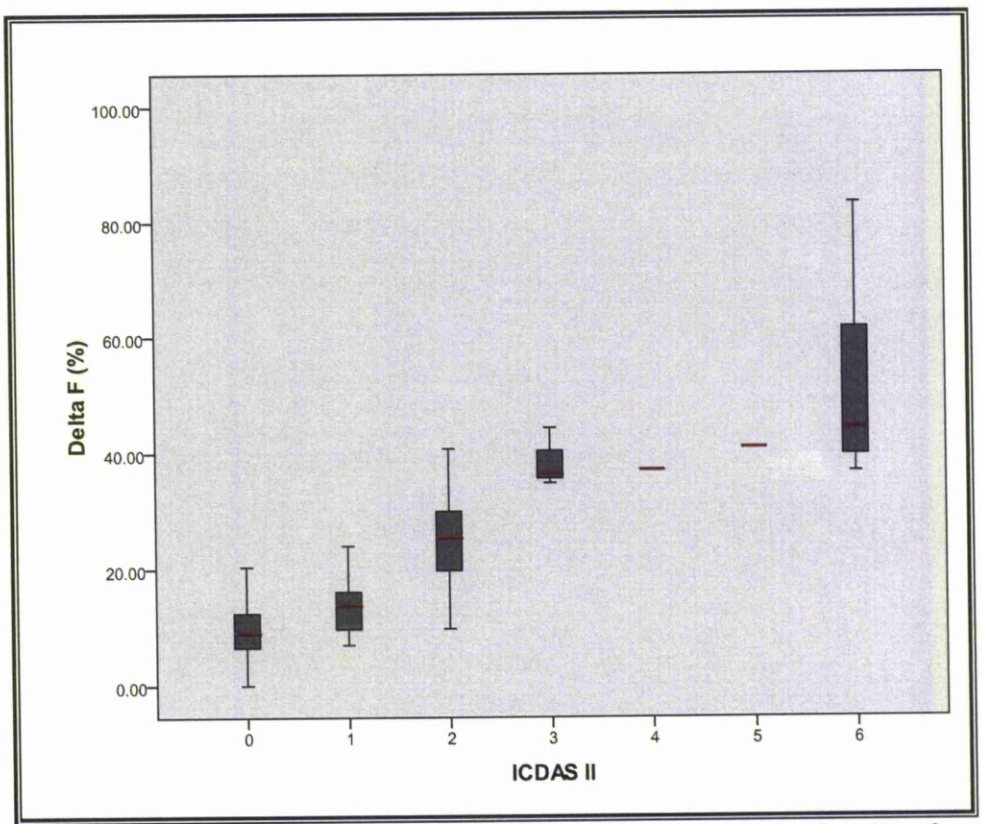


Figure 3.34: Graph showing ICDAS II scores against ΔF (%) on distal surfaces.

3.6.5.2 ICDAS II with ΔQ

Results in Figure 3.35 show that ΔQ correlated positively with the ICDAS II visual index more in its early stages (ICDAS II 0, 1, 2 and 3). The correlation coefficient between ICDAS II scores and ΔQ (0.690) was significant at the 1% level.

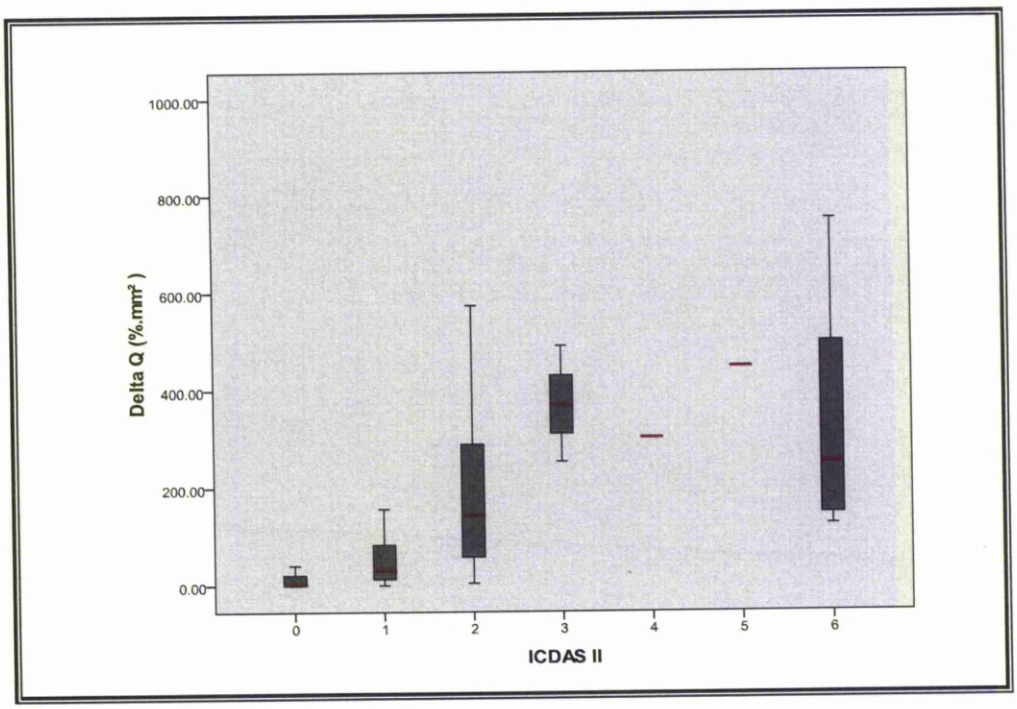


Figure 3.35: Graph showing ICDAS II scores against ΔQ (%.mm^2) on distal surfaces.

3.6.5.3 ICDAS with ΔR-QLF and ΔR-Morita.

The results presented in Figure 3.36 demonstrate that there was a good correlation between ICDAS II and ΔR-QLF as well as ΔR-Morita on distal surfaces of the teeth in the early stages of ICDAS II. The images taken with the Morita camera showed better correlation with ICDAS II than the QLF. The increase in ΔR-Morita values were more strongly correlated to the increase in ICDAS scores (0.713) than the ΔR-QLF values (0.650).

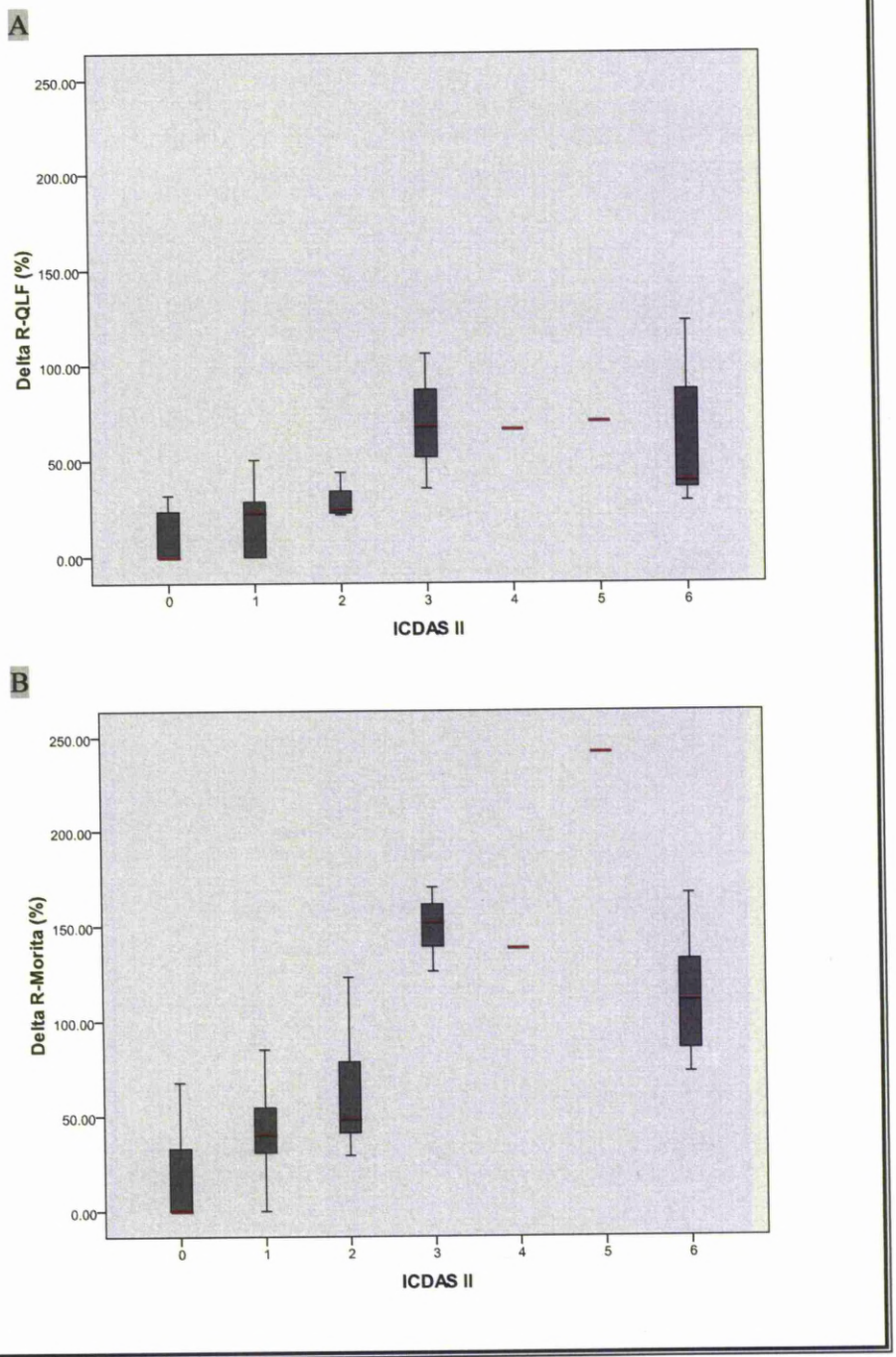
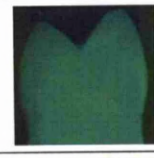
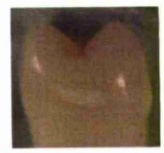
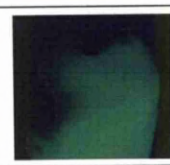


Figure 3.36: Graph showing ICDAS II scores against (A) ΔR-QLF (%), (B) ΔR-Morita (%) for distal surfaces.

3.6.5.4 QLF distal surfaces index examples.

After analysing all the data of QLF parameters on the distal surfaces, the index derived and examples are shown in Table 3.13.

Table 3.13: Examples of QLF distal surfaces index and their corresponding ICDAS II classifications.

ICDAS II Classification			QLF Classification system			
				ΔF (%)	ΔR (%)	QLF Index Score
Code	Description	Example	Example	Index	Index	
0	Sound.			-0.5-10	0-20	QLF 0
1	Brown/white first visual change in enamel.			10.5-15	21-35	QLF 1
2	Brown/white distinct visual change in enamel.			15.5-25	36-60	QLF 2
3	Localised enamel break-down.			25.5-30	61-78	QLF 3
4	Underlying shadow.			30.5-35	79-92	
5	Distinct cavity.			35.5-45	93-99	QLF 4
6	Extensive cavity.			>45.5	>99	

3.6.6 Correlation of ΔF, ΔQ, ΔR-QLF and ΔR-Morita with ICDAS II scores on all the surfaces

Results presented in Table 3.14 show the correlation of ΔF, ΔQ, ΔR- QLF and ΔR-Morita with ICDAS II scores on occlusal, buccal, lingual, mesial and distal surfaces of the teeth.

Table 3.14: Correlation of ICDAS II scores on all the surfaces with ΔF, ΔQ, ΔR.

ICDAS II scores	ΔF	ΔQ	ΔR (QLF)	ΔR (Morita)
Occlusal	0.772	0.710	0.634	0.738
Buccal	0.701	0.733	0.481	0.588
Lingual	0.700	0.716	0.603	0.590
Mesial	0.690	0.687	0.490	0.601
Distal	0.750	0.690	0.650	0.713
Mean correlation	0.722	0.707	0.571	0.646

Correlation was significant at the 1% level.

3.6.7 Correlation of ΔQ, ΔR-QLF and ΔR-Morita with ΔF values on all the surfaces

Results presented in Table 3.15 show the correlation of ΔQ, ΔR- QLF and ΔR-Morita with ΔF on occlusal, buccal, lingual, mesial and distal surfaces of the teeth.

Table 3.15: Correlation of ΔF values on all the surfaces with ΔQ, ΔR.

ΔF	ΔQ	ΔR (QLF)	ΔR (Morita)
Occlusal	0.700	0.636	0.758
Buccal	0.888	0.536	0.631
Lingual	0.900	0.603	0.724
Mesial	0.896	0.563	0.615
Distal	0.870	0.668	0.674
Mean correlation	0.850	0.601	0.680

Correlation was significant at the 1% level.

3.7 Discussion

In order to guide researchers and clinicians in the appropriate selection and use of new technologies, latest methods need validation and evaluation with established techniques and have to be practical and feasible for clinical application and use.

One of the rationales of this project was to develop and validate an interpretative index for QLF on occlusal surfaces of the teeth as well as on smooth surfaces (buccal, lingual, mesial and distal) and to facilitate its practice by the dental clinician and students to understand the information produced by its software. It is particularly important to improve and enhance the detection of dental caries in its early stages to allow intervention of preventive regimes in order to reduce the degree of invasive restorative treatment. The diagnosis of early caries lesions can be considered as the foundation stone of cost effective health care delivery and quality of dental care. Since caries diagnostic research is ultimately aimed at maintaining and improving oral health care, it is the researchers' aim that knowledge and tools are transferred and applicable for clinical dentistry. Therefore, there should be an equilibrium between the effort in caries diagnostic research and the application of research results in dental health care (Verdonschot et al., 1999).

Conventional clinical visual methods of caries detection of macroscopically integral occlusal surfaces have been shown to have relatively poor sensitivity values, well below 30% (Lussi, 1993; Wenzel et al., 1991b).

The changing disease patterns with a general caries decline associated with a relatively high number of non-cavitated caries lesions was one of the reasons why the International Caries Detection and Assessment System II (ICDAS II) was developed recently (Pitts, 2004a). This comprehensive visual examination method which records early noticeable signs of the carious process such as opacities, brown discolorations, enamel breakdowns or micro-cavities without clear cavity was used. These visual signs have proven to be good indicators of the presence of enamel and/or dentine lesions (Ekstrand et al., 1998; Ekstrand et al., 2001; Nyvad et al., 1999). Previous studies have shown that the Spearman's correlation coefficient for 5 points visual examination was ranged between 0.78-0.93 (Ekstrand et al., 1997). In this study, Spearman's correlation coefficient for ICDAS II with ERK histological scores was 0.800 and it was generally excellent; representing a strong relationship between two variables. Intra-examiner reproducibility for the visual ICDAS II examination in this study was, kappa = 0.81. To date little has been published on the visual ICDASII system; on the other hand, a recent study which also used the ICDAS II system found kappa values of 0.79 (Jablonski-Momeni et al., 2007) and 0.78 (Jablonski-Momeni et al., 2008), were quite similar. However other related scoring systems have been used previously. In one study an intra-examiner kappa value of 0.90 was obtained using an eight-point scoring system for diagnosing occlusal caries (Ekstrand et al., 1995) Using a five-point scoring system, 0.81 for intra-examiner agreement was observed (Ekstrand et al., 1997). In another study using the same system, kappa values of 0.62 for intra-examiner reproducibility (Reis et al., 2004) and 0.69 (Braga et al., 2009) were achieved. The reproducibility achieved with the ICDAS-II seven-point scoring system in this

study compared favourably with those cited using the same system. The sensitivity and specificity of ICDAS II with histological score was high. Using the ICDAS II and ERK histological threshold allowed comparison with previous work (Ekstrand et al., 1997) where higher sensitivity (0.94) and specificity (0.89) were obtained. In the most recent study where ICDAS II and ERK histological threshold used (Jablonski-Momeni et al., 2008) a mean sensitivity of 0.60 and a mean specificity of 0.90 were obtained. Intra-reproducibility of histology scores in this study was of kappa= 0.79 which was a substantial agreement.

In this study ICDAS II visual examination was used to develop a visual code system (VCS) previously described in section 3.3.3, the possible advantages of using this code system are that it can be used during dental charting (by the dental nurse or dental student) giving a general picture about the situation of the teeth especially the tooth under investigation. If it used routinely by dental care professional for each tooth surface, it will encourage the use of the ICDAS II system so that the dental professionals will look closely for the first visual changes on tooth surfaces as this system meets the requirements of validity and reliability (Ismail et al., 2005). The data presented by Ismail and his group showed that for the most frequent disease condition, pit-and-fissure caries, the system has correlation strength. Furthermore, ICDAS II has a good reliability even when used by examiners who had no earlier skill in epidemiological dental assessment (Ismail et al., 2007). In addition the VCS may promote dental professionals to use practical and clearly defined measures for clinical visual caries detection which may

improve the sensitivity in individual assessors' reading for different lesion's characteristics.

Dentists are among the most prolific prescribers of radiographic imaging for clinical examinations. Radiography is a well-accepted and fundamental part of diagnostic and management procedures (Pretty and Maupomé, 2004). Radiography can boost low sensitivity for conventional clinical visual methods, however, it is linked with the unavoidable risks of ionising radiation (Ferreira Zandona et al., 1998a) and that the radiographs have a higher specificity than sensitivity (Dove, 2001) which was confirmed by recent study as 35%, 60% (sensitivity and specificity respectively) in enamel caries and 91%, 95% (sensitivity and specificity respectively) in dentinal caries (Bader et al., 2001). In this study radiographs correlated with histology on occlusal surfaces (0.591) which is similar to previous findings (0.54) (Wenzel and Fejerskov, 1992) and (0.77) (Ekstrand et al., 1997). This confirms that this technique has been shown to perform poorly in detection of caries especially caries on the occlusal surfaces. On the other hand, the visual scoring index correlateed less well with radiographs (0.670 on occlusal, 0.510 on buccal, 0.590 on the lingual, 0.610 on mesial and 0.510 on the distal surfaces).

A standard analysis technique was employed to reduce the subjectivity. QLF parameters which linked with histology are the most useful comparison if early detection of dental caries, early intervention and minimal dental tissue destruction is to be achived. ICDAS II correlated best on all surfaces with ΔF then with ΔQ. ΔF

values obtained from the analysis of the occlusal surfaces of the teeth correlated with the histology (0.753) this correlation was significant at the 1% level.

Red fluorescence (RF) can be seen anywhere in the mouth but more often in plaque retention sites, and the absence of RF is an indicator of a clean cavity (Lennon et al., 2003). However, bacteria are not recognised to fluoresce themselves, although they were reported to produce by products as a result of their metabolism which is orange-red fluorophores (König and Schneckenburger, 1994). RF of caries has been shown to be the result of synthesising porphyrins and metalloporphyrins by oral bacteria (König et al., 1998), particularly Gram-negative anaerobes, which are more numerous in mature than in early plaque (Coulthwaite et al., 2006).

ΔR-QLFand ΔR-Morita correlated less well on all surfaces with ICDAS II than the correlation of ΔF and ΔQ with ICDAS II on all surfaces. However, correlation of ΔR-QLF and ΔR-Morita with ΔF on all surfaces was better. It has been suggested that lesions that show red fluorescence were active and have the potential to develop into more advanced lesions since they contain active bacteria. Hence, red fluorescence should be used as secondary measure only, to indicate bacterial infection particularly in advanced lesions.

In this *in vitro* study it was observed that there was a reduction in the red fluorescence in most of the surfaces for the lesion with ICDAS II scores 5 and 6. The possible reasons for this are firstly, the nature of the *in vitro* study which investigated teeth collected from another country. It may be that the transportation

with the change in the temperature and light exposure may had an effect. Secondly, when working under laboratory conditions, several variables may have had an effect, for example room temperature, humidity and storage may exert a bacteriostatic effect. However, some bacteria may succeed in surviving under low temperature situations (Francescut et al., 2006). In addition the length of time since extraction may cause differences in results. Extracted human teeth are infected with microorganisms (Schulein, 1994) and therefore, it is essential that these teeth are disinfected before any laboratory experiment is performed. A variety of procedures to miminmise bacterial contamination have been used for tooth storage, one of which is thymol (Aquilino et al., 1987; Boyd, 1976; Causton and Johnson, 1979; Retief et al., 1989). Moreover, storage conditions have been reported as a cause of changes in fluorescence values (Francescut and Lussi, 2000). Investigations in their laboratory showed that the fluorescence value of occlusal surfaces decreased rapidely in the first 5 months of storage in thymol or formalin and levelled off thereafter. The experiments reported in that study were undertaken after this initial period and an influence of the storage solution during the experiment can therefore be ruled out (Lussi and Francescut, 2003).

For some ΔQ on the occlusal and distal surfaces, a deviation was noted with ICDAS II scores 4, 5 and 6. This can be readily explained firstly, from the fact that high scattering properties of carious enamel causes fluorescence loss (as observed by QLF). Missing tissue does not cause scattering, and hence the fluorescence pattern is determined solely by the underlying tissue. Secondly, that the number of teeth from the whole study sample fell on ICDAS II scores (4, 5 and 6) on buccal,

lingual, mesial and distal were less than 5 in each while the majority of the sample distributed between ICDAS II 0, 1, 2 and this suggested that ΔF is the best QLF parameters to link with ICDAS II scores.

A preliminary index was prepared by analysing all the results and a QLF score developed with relation to histology and ICDAS II scores. In addition, for the occlusal surface the derived QLF indices presented maximum fluorescence loss and histology scores. This has been carried out in this study to reflect and to demonstrate its importance since it was incorporated in the older version of the software but not the newer version.

A previous study (Higham et al., 2003) compared QLF ΔF values with a 5 point visual index (Ekstrand et al., 1997) whereas in the current study a 7 point ICDAS II classification was used. The same histological and radiographic index was employed both studies. A high level of correlation was observed between radiographs on the occlusal surfaces and the gold standards (0.83) (Higham et al., 2003). This result was unexpected since this technique performed poorly in occlusal caries detection although it was substantially higher than that obtained from an *in vitro* study conducted in 1992 (0.54) (Wenzel and Fejerskov, 1992). In this study the correlation was 0.591 and the correlation between the histology and ΔF was similar to that observed by Higham and co-workers (Higham et al., 2003). The index developed by the group was not validated and it did not agree with the subdivision of ΔF values for each histological value observed in the index developed in the current study which used a larger sample size, a greater variety of

techniques, newer version of QLF software and compared with other more recent indices such as ICDAS II.

It has been stated by the author that " the index may require revision in order to maximise these values" (Higham et al., 2003) and that is what has been achived in the current work. This is the first study to develop a QLF index for the smooth surfaces; therefore there is no data to compare the current findings with.

To confirm the quantitative nature of QLF measurements in relation to an accepted "gold standard", histology measurements were made. This index was found to have a high sensitivity and specificity with histology. It can be seen that the QLF technique performed better in the early stages of dental caries and therefore, its importance in early diagnosis and detection is apparent. These findings suggest that the device is usful to distinguish, separate and sort early lesions from sound tissues, where visual detection is difficult and this reflect the clinical potential of this technology.

In 1991, a detailed review of quantitative methods to determine mineral changes recommended the use of radiographic methods to quantify mineral loss in whole teeth. The interest in radiation techniques is due to the ability of X-rays to travel through matter without destroying the specimens (Ten Bosch and Angmar-Mansson, 1991). Currently, TMR is considered as the 'gold standard' for the determination of mineral loss in experimentally made incipient lesions.

The Transverse Microradiography (TMR) technique requires the preparation of planoparallel transverse thin sections from calcified tissue; these sections must allow X-rays to be absorbed only partially and in a simple relationship to the mineral content. The method has been used for the assessment and confirmation of other recently developed caries diagnostic techniques (Damen et al., 1997). A key disadvantage of microradiography lies in its superimposition effect, such that any non-uniformity detected in the direction of the X-ray beam is lost due to this effect. In addition, specimens need to be physically cut into thin sections which is time consuming and destructive (Gao et al., 1993).

Difficulties arise when the TMR technique is used since there is often an irregular or interrupted surface layer on the specimen. In this study it was observed that sections from human teeth with incipient lesions often had a missing surface layer and because of the curvature of the natural surface of teeth; some of caries lesion may not be included in the scan area. It was therefore very difficult to scan the whole natural carious lesion.

The TMR technique is very sensitive and requires a lot of careful preparation and is time consuming. It was found in a study conducted in 2001 that the correlation of the QLF method with integrated mineral loss in natural lesions was lower than that of artificial lesions, which may be explained by the fact that natural lesions are more difficult to analyse since they are less uniform and may may have increased lesion depth (Shi et al., 2001). Loss of information between sections is also a disadvantage because by using this technique; it only provided a snap shot of the

lesion at a particular stage in its dynamic development (Huang et al., 2007). The main objective for using this technique in the study was only to illustrate that TMR images showed surface softening and subsurface lesion and served to confirm the observation observed by the QLF. Correlation between QLF and TMR data in a recent study indicated the validity of the former as a measure of mineral content (Higham et al., 1991).

The most appropriate method to be used as a 'gold standard' was found to be microscopic inspection for lesion depth validation by stereomicroscopy of sections. This agrees with what have been found in a recent study (Huysmans and Longbottom, 2004) which concluded that for quantifying lesions depth in larger lesions the exposure difficulties have not been resolved and for lesions on occlusal surfaces, the consequence of uneven morphology cause disadvantages with this radiographic technique. Sectioning of the specimen is necessary for all histological gold standards and this causes a considerable part of the section to be lost, typically 120-250 μm per slice (Huysmans and Longbottom, 2004). If sectioning is to be evaded, X-ray microtomography would be the method of the choice.

The use of the microtomographic technique (MCT) in dentistry is relatively new and still needs to be investigated and fully evaluated, in order to find its limitations and advantages. MCT is used to measure the mineral concentration in calcified tissues. It has the benefit of being a non-destructive technique capable of obtaining quantitative measurements of mineral concentrations mass samples by employing the linear attenuation coefficient. The linear attenuation coefficient (μ) is the

probability that an X-ray or gamma-ray photon will interact with the material it is traversing, per unit path length travelled. It is usually reported in units of cm-1 and depends on the photon energy, chemical composition and physical density of the material (Amaechi, 2004; Amaechi and Saldaña, 2004).

It has been reported that micro-CT requires no preparation in terms of cutting cross-sections (Bergmans et al., 2001; Hahn et al., 2004), and this enables longitudinal experiments to be conducted in three-dimensional studies thereby overcoming the disadvantages of microradiography. However, there are some disadvantages. Scanning is exteremely time consuming (Anderson et al., 1996) and the system has limitations with respect to energy selection, scans artefacts, calibration and costs (Clementino-Luedemann and Kunzelmann, 2006). It was found in the current study that as the dental caries increased in its severity the percentage change in mineral density by micro-CT increased, however no significant correlation was found with ΔF.

QLF images of tooth sections were found to provide a good general picture regarding the caries disease progression into the dental tissues with the advantages of the contrast and the enhanced visual appearance. It is clear to distinguish whether the lesion is limited to the enamel, or extends into dentine and the degree of proximity to the pulp. Moreover, in some images red fluorescence has been observed which may indicate bacterial invasion. The correlation of histology with the average fluorescence loss of tooth's sections following imaging by QLF and analysis was 0.601. QLF was also useful to confirm the level of dental caries

involvement into the tissue in histological sections without the need for more materials and extra steps.

The current QLF system is adapted to be used in the clinic since it is compact, not difficult to master, easy to clean and maintain infection control between patients. This technology is believed to be motivative for dental patients as it shows the original dental situation, improvement or otherwise in teeth over time in clear visual way which easily understandable to patients (Kuhnisch and Heinrich-Weltzien, 2004).

Scientific evidence that the early stages of caries can be arrested and possibly reversed if the caries challenge is modified or removed (reduced cariogenic diet, improved oral hygiene) or if the protective factors are increased (fluoride in various delivery modalities, increased salivary flow for those with decreased salivary flow), provides strong support for taking a more conservative approach to the management of non-cavitated dental caries. A demanding issue challenging dentists' on a daily basis is deciding whether preventive intervention or a combination of preventive and restorative intervention is required. For this reason a device such as QLF with its relatively high sensitivity and specificity is needed. However, it should not lead to the over diagnosis of caries and possible overtreatment but help clinicians to detect caries at a threshold that requires preventive intervention.

3.8 Conclusion

The results of this study suggest that QLF is appropriate for use in identifying dental caries and demineralisation in early stages on the occlusal as well as smooth surfaces. It is widely recognised that progression of an early carious lesion may become arrested or that the lesion may further demineralise or remineralise. Current practice in dentistry has evolved to offer a minimally invasive approach, in which caries is, managed by deferring operative involvement for as long as possible. The focus is on maximum conservation of demineralised, non-cavitated enamel and dentine. Therefore, QLF will have a significant future role in modern dental caries management. It can be concluded from the present investigation that, QLF was able to identify caries on all tooth surfaces and differentiate between lesions of varying severity particularly during the early stages of the disease while the Morita images give information regarding red fluorescence only. Moreover, as QLF is sensitive it may be used to examine the occlusal surfaces without the need for probing and avoid the risks associated by using the dental explorer. It is anticipated that QLF will be a valuable tool in routine clinical practice and it may also reduce the patient's exposure to ionising radiation and allow more frequent longitudinal monitoring of patients teeth since it offers a non- radiographic method to identify dental caries in its early stages.

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CHAPTER 4

THE *IN VIVO* STUDY 1

Development of Caries Indices Using Quantitative Light-induced Fluorescence (QLF) *in vivo*.

4.1 Introduction

Dental caries still remains one of the most prevalent diseases affecting humans (Schulte et al., 2008). Clinical assessment of early dental caries is based on subjective qualitative methods (Eccles, 1979). Transillumination, radiography and visual examination can only detect caries in more advanced stages and offer no quantitative data of the early changes in mineral contents of the enamel (Mistry and Grenby, 1993).

Overall, *in vitro* representations are mechanistically limited in several areas. Firstly, insufficient imitation of biological aspects of dental caries. Secondly, difficulties in matching conditions happening *in vivo* and finally, artifacts related to the enamel and/or dentine slabs choice. Therefore, it is almost unfeasible for *in vitro* models to sufficiently simulate the complex and varied intra oral conditions (White, 1995).

To date several advanced methods which enable early detection and quantification have been introduced and tested. However, techniques that allow visualisation of mineral changes in the caries process, such as Quantitative Light-induced Fluorescence (QLF), appear to have the greatest potential in this regard (De Josselin de Jong et al., 1995; Stookey et al., 1999).

Quantification of data from the process of disease has long formed the basis for special investigations. Quantifying methods have benefits which include minimising human error, follow-up comparisons and greater reproducibility; thus

facilitating computerised data handling, storage, and retrieval together with longitudinal monitoring. Optical methods enhance the contrast between the sound and carious/demineralised dental tissues; thus it is helpful for quantification. Likewise, quantification of depth is more practical in dental caries diagnosis for the reason that vision estimates contour and size much better than depth. Optical quantification methods have the advantage of simplicity, easy understanding, easy acceptance and reduced subjectivity (Ten Bosch, 1987).

Studies evaluating the tooth in its natural environment are ideal as the clinical relevance and application of such studies tend to be more valid. In the daily work of a dentist, the use of visual methods for assessing dental caries are reinforced from day to day since diagnosis as well as restorative treatment decisions depend primarily on interpretation of visual information gathered. The advantage of optical diagnostic methods is, therefore, that they have close connection with visual observations and visual representations existing in the brain (Ten Bosch, 1987). Investigation is required to facilitate and optimise the use of these devices in every day dental practice. Therefore, research not only helps to prove the validity of outcomes obtained from the new technologies, but also equally vital, provides guidelines for their clinical use. Using these devices could have a clear impact on the daily approach to caries diagnosis and management.

4.2 Aims and objectives

1-The overall aim of the study was to develop and validate a clinically applicable index for QLF that relates green fluorescence loss (ΔF and ΔQ) and extent of red fluorescence (ΔR) to ICDAS II caries scores for occlusal, buccal and lingual surfaces.

2- To validate *in vivo* the QLF occlusal caries index previously developed *in vitro* that relates green fluorescence loss to ICDAS II and histology.

3- To develop an interpretive index for clinical use either for prevention and/or restorative treatment.

4- To evaluate the use of QLF *in vivo* for approximal caries and for "hidden caries" diagnosis.

4.3 Subjects and settings

4.3.1 Ethical approval

All the necessary documents and forms were completed, the required supplementary documents provided (University of Liverpool sponsorship letter, Reference: 000214) (Appendix 2) and then, submitted to The Liverpool Adult Local Research Ethics Committee. Ethics approval was obtained (Reference number: 07/Q1505/23) (Appendix 3) and NHS Research and Development approval granted to start the study (Appendix 4).

4.3.2 Recruitment

83 subjects attending an outpatient oral surgery clinic in the accident and emergency department at The University of Liverpool Dental Hospital were recruited over a period of seven months. Subjects underwent the routine clinical examination and investigations appropriate to the management of their clinical condition.

4.3.3 Subject information sheet and consent

Each patient received a copy of subject information sheet (Appendix 6) early in the morning when they first reported to the accident and emergency reception. Posters describing the study were on display in reception and waiting areas (Appendix 5). Patients were informed that their names and teeth would be anonymised prior to initiation of study procedures. It is essential for good clinical practice to obtain the subjects consent before the beginning of any dental research work (Smeeton, 2005). Therefore, positive informed written consent, in which the patient formulated a specific decision, to enter the research study was obtained and the record kept securely (Appendix7).

4.3.4 Criteria for selection:

4.3.4.1 Inclusion criteria were:

A subject was enrolled in the study if the following criteria were met:

1. Adults 18-75 years of age,

2. Presence of at least one natural posterior tooth,
3. Good general health and condition.

4.3.4.2 Exclusion Criteria

A subject could not be enrolled in the study if any of the following criteria were met:

1. Edentulous.
2. Subject was pregnant (based on oral interview only).
3. Concurrent participation in research study or within 30 days of participation in another trial.

4.3.4.3 Sample size

A sample size of 350 teeth was selected. Data from a previous study conducted *in vitro* showed that ΔF measurement from QLF analysis correlated with the histological index at r =0.853. For the present study, the minimum sample size required to estimate the Spearman's correlation coefficient with a given accuracy was calculated. The width of the 95% confidence interval was set to 0.2 (e.g. 0.6 to 0.8). This gave a minimum sample size of 334 teeth. A previous study conducted by Lussi and his group used a sample size with a total of 332 teeth (Lussi et al., 2001).

4.4 Procedures

4.4.1 Clinical investigation

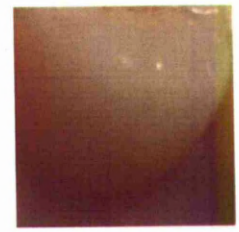
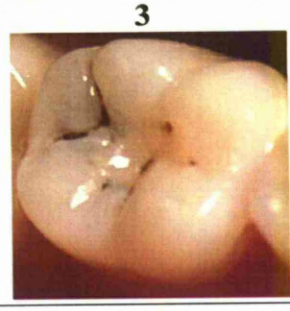
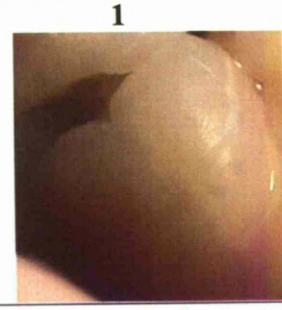
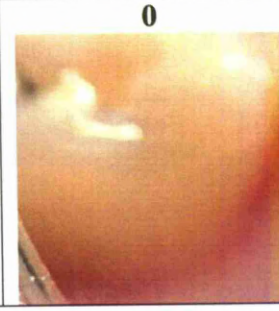
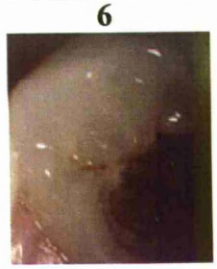
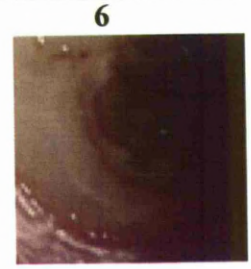
The teeth that had fluorosis or other developmental imperfections (which could appear hypomeneralised) were excluded. Those teeth considered by clinician to require further investigation were subjected to appropriate radiographic examination. After the radiographs were obtained, a dental prophylaxis was performed for the teeth under investigation providing that the patient was able to tolerate the procedure. Some patients presented with swelling or severe pain and were unable to bear the use of the cold water necessary during prophylaxis.

Teeth were cleaned with a rotating rubber polishing cups (Screw type, Minerva Dental Limited, Cardiff, UK), paste (NUPRO® Prophylaxis Paste, medium orange, DENTSPLY, USA) and water. The patient was asked to rinse with water and the teeth were then dried for 5 seconds. For those patients who could not tolerate the procedure due to their pain, cleaning was carried out by using wet cotton wools. All the examinations were conducted under standardised conditions using a dental unit with a light, dental mirror, compressed air and evacuation facilities (KaVo Dental, Biberach/Riss, Germany). ICDAS II clinical visual examination was carried out for occlusal, buccal and lingual surfaces of the teeth and the information obtained entered on clinical charts specially designed by the researcher for this study (Appendix 8). Visual assessment resulted in a 3 digit score (visual code system, VCS) developed specifically for this study. For each tooth VCS consists of

a single digit score using the ICDAS II criteria for each surface in the sequence of occlusal, buccal and lingual (OBL) Table 4.1. White light digital images were taken for the occlusal, bucal and lingual surfaces of each tooth by the using a digital camera (Nikon D200 SLR Camera, Japan) with the help of cheek/lip retractor and intra-oral mirror. In the same way QLF images (Inspektor Pro 2.0.0.39, Inspektor Research System BV, Amsterdam, The Netherlands) and in addition, Morita- Penviewer Camera (J. Morita MFG. CORP., Tokyo, Japan) images were taken for each tooth surface (O, B and L).

A digital image of the radiograph was taken while it was on the X-ray viewer and its radiographic index recorded in the clinical chart using a five-point scale (Ekstrand et al., 1997). Following extraction, teeth were placed in a sealed tube containing thymol crystal (0.1% w/v) (GPR ^{TM} , Poole, England) and water, labelled with the subject's code and transferred to the laboratories of the research wing of the School of Dental Sciences.

Table 4.1: Examples of teeth classified using the ICDAS II and the visual code system.

Tooth's Study Code.	Visual Examination Index (ICDAS II).		
	Occlusal	Buccal	Lingual
CI 49	**1** 	**0** 	**0** 
	Visual Code System (VCS) **100**		
CI 69	**3** 	**1** 	**0** 
	Visual Code System (VCS) **310**		
CI 70	**6** 	**4** 	**6** 
	Visual Code System (VCS) **646**		

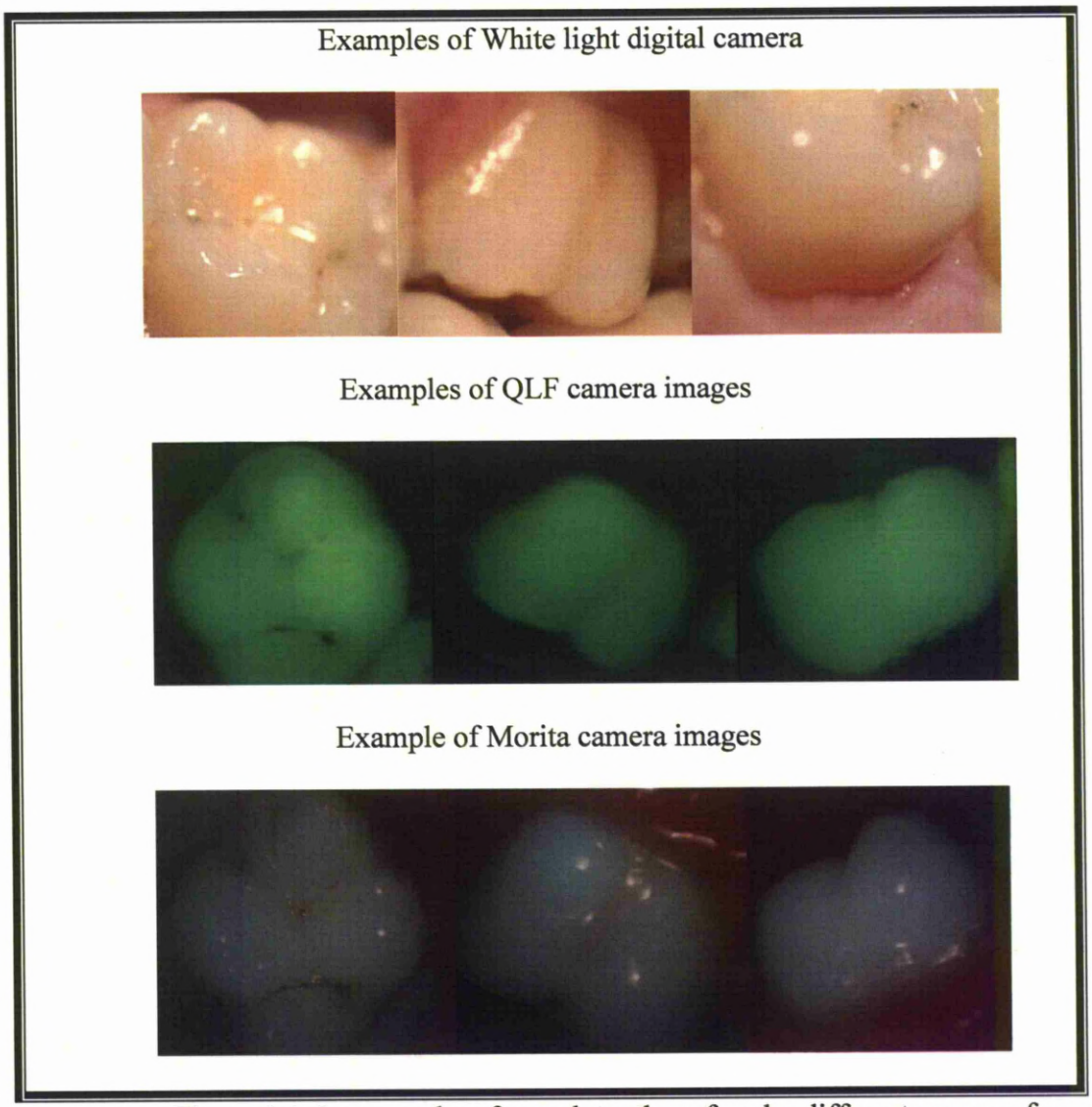


Figure 4.1: Images taken for each tooth surface by different cameras for one tooth.

4.4.2 Laboratory investigation

To verify the quantitative nature of light-induced fluorescence measurements in relation to accepted "gold standard" histology, initially, extracted teeth were thoroughly rinsed with water and then cleaned with pumice (Associated Dental

Products LTD, dental material manufacturers, Purton, Swindon, Wilts), wet-and-dry paper (grit size of 400 μm) and a handpiece (KaVo EWL, Germany) with brush (junior cup tooth polishing brushes bristle RA, Minerva Dental Limited, Cardiff, UK). The root component was then removed horizontally at the enamel cementum junction (ECJ) by a handpiece with a diamond disc (Skillbond, UK). The roots were discarded and the crown portion retained, then each crown was sectioned using a diamond wire saw (Well Wire Saw, The Precision Diamond Wire Saw Series 3, Switzerland) in a mesial to distal orientation through the crown of the tooth to give two halves. Each half examined under a steromicroscope (SMZ 10, Nikon, Japan) and scored using a five-point ranked histological scoring system by which the depth of the lesion was assessed (Ekstrand et al., 1997). Finally, an image of the half of the tooth indicating deeper carious involvement was taken using a digital microscopy camera (Moticam 2300, 3.0 M Pixel, China) and each section was scored twice.

4.5 Analysis

4.5.1 QLF analysis

Three QLF images for each tooth (occlusal, buccal and lingual surfaces) were taken and stored in the QLF PC, giving a total of 1050 images. Each image was analysed for white spots (WS) and red fluorescence (RF) using QLF software (Inspektor Pro 2.0.0.39, Inspektor Research System BV, Amsterdam, The Netherlands). A standard analysis technique was employed to reduce the bias. For

every lesion, the ΔF (%), the area of the lesion (mm^2) ΔQ, ΔR (%) and RF (mm^2) areas were calculated using the software at the 5% threshold level. After completion of the QLF analysis, a total of 2100 images were produced from this analysis, exported to word files and stored, example of which are shown in Table 4.2. The data were decoded and entered into SPSS for statistical analysis.

4.5.2 Morita analysis

Three Morita images were taken for each tooth (in the same way as QLF images were taken). The images were stored on the same QLF PC. Each surface image was analysed by the use of QLF software for red fluorescence, with a total of 1050 images analysed. ΔR and RF area values were recorded for each tooth surface, with another 1050 images produced after analysis, exported to word files and stored. The data were decoded and entered into SPSS for statistical analysis.

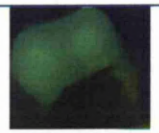
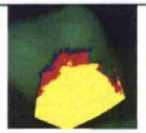
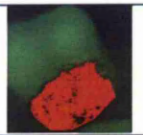
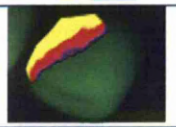
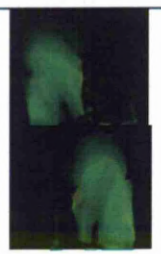
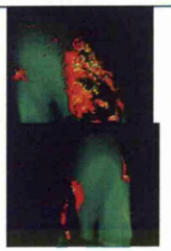
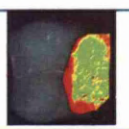
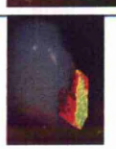
Case Number	QLF Images		QLF Analysis		Analysis Values		Index Score	
			WS	RF				
(CI 51) QLF images	Occlusal				ΔF[%] ΔQ[%.mm²] ΔR[%] Ws Area [mm²] RF Area[mm²]	57.30 1080 96.5 32.8 24.6	ICDAS II 	**6**
	Buccal				ΔF[%] ΔQ[%.mm²] ΔR[%] Ws Area [mm²] RF Area[mm²]	45.2 693 25.2 17.5 0.24	ICDAS II 	**5**
	Lingual				ΔF[%] ΔQ[%.mm²] ΔR[%] Ws Area [mm²] RF Area[mm²]	54.2 507 65.7 9.37 6.14	ICDAS II 	**6**
Histological sections examined with QLF	First Half Second half				ΔF[%] ΔQ[%.mm²] ΔR[%] Ws Area [mm²] RF Area[mm²] ΔF[%] ΔQ[%.mm²] ΔR[%] Ws Area [mm²] RF Area[mm²]	83.8 2860 146 34.2 15.9 83.8 2770 63.8 33.1 3.55		Histological: 4
Morita Images and analysis	Occlusal				ΔR[%] RF Area [mm²]	243 27		Radiographic: 4
	Buccal				ΔR[%] RF Area[mm²]	302 9.34		
	Lingual				ΔR[%] RF Area[mm²]	148 8.97		

Table 4.2: An example of data collection and gathering prepared for each tooth.

4.6 Statistical method

The data were statistically analysed using SPSS for Windows software (17.0). The data for ΔF, ΔQ, ΔR for QLF and ΔR for Morita were examined and compared with ICDAS II scores. Scatter diagrams and box plots were used as these were considered the most useful starting point in investigating the correlation between the two variables and offer both a visual and statistical means to test the strength of a potential relationship. In addition, box plots were also used to demonstrate the key statistical variables of the data set.

Spearman's Rank correlations (Spearman's rho) were used for correlation between different variables and specificity and sensitivity were also calculated. The quality of the intra-examiner reproducibility was calculated using kappa values. For each ICDAS II score the confidence intervals for green fluorescence loss and red fluorescence level was determined.

4.7 Results

The spearman's correlation coefficients for a lesions average green fluorescence loss ΔF, ΔQ, ΔR for QLF and ΔR for Morita with corresponding ICDAS II scores are presented for the occlusal, buccal and lingual surfaces of the teeth. Furthermore for the occlusal surface the histology was presented and correlated with the variables.

4.7.1 Occlusal Surface

4.7.1.1 ICDAS II with Delta F.

The results presented in Figure 4.2 show that ΔF at the 5% threshold level correlated positively with the ICDAS II visual index, as the ICDAS II increased the average green fluorescence loss increased. Therefore, as the severity of dental caries increased the fluorescence loss increased.

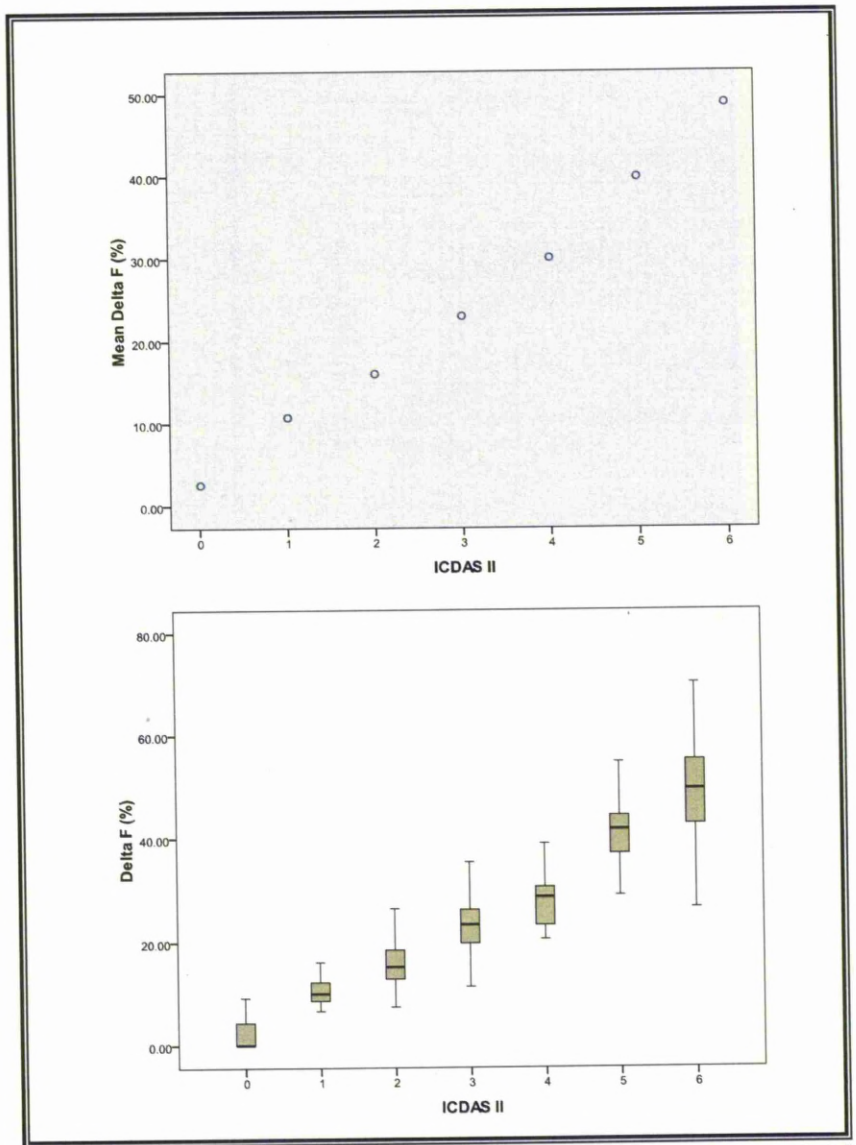


Figure 4.2: Scatter plots of the mean and box plot for ΔF against ICDAS II for occlusal surfaces.

4.7.1.2 ICDAS with Delta Q.

The results presented in Figure 4.3 show that ΔQ (area×ΔF) correlated positively with the ICDAS II visual index. The ICDAS II scores increases linearly as the tooth loses mineral.

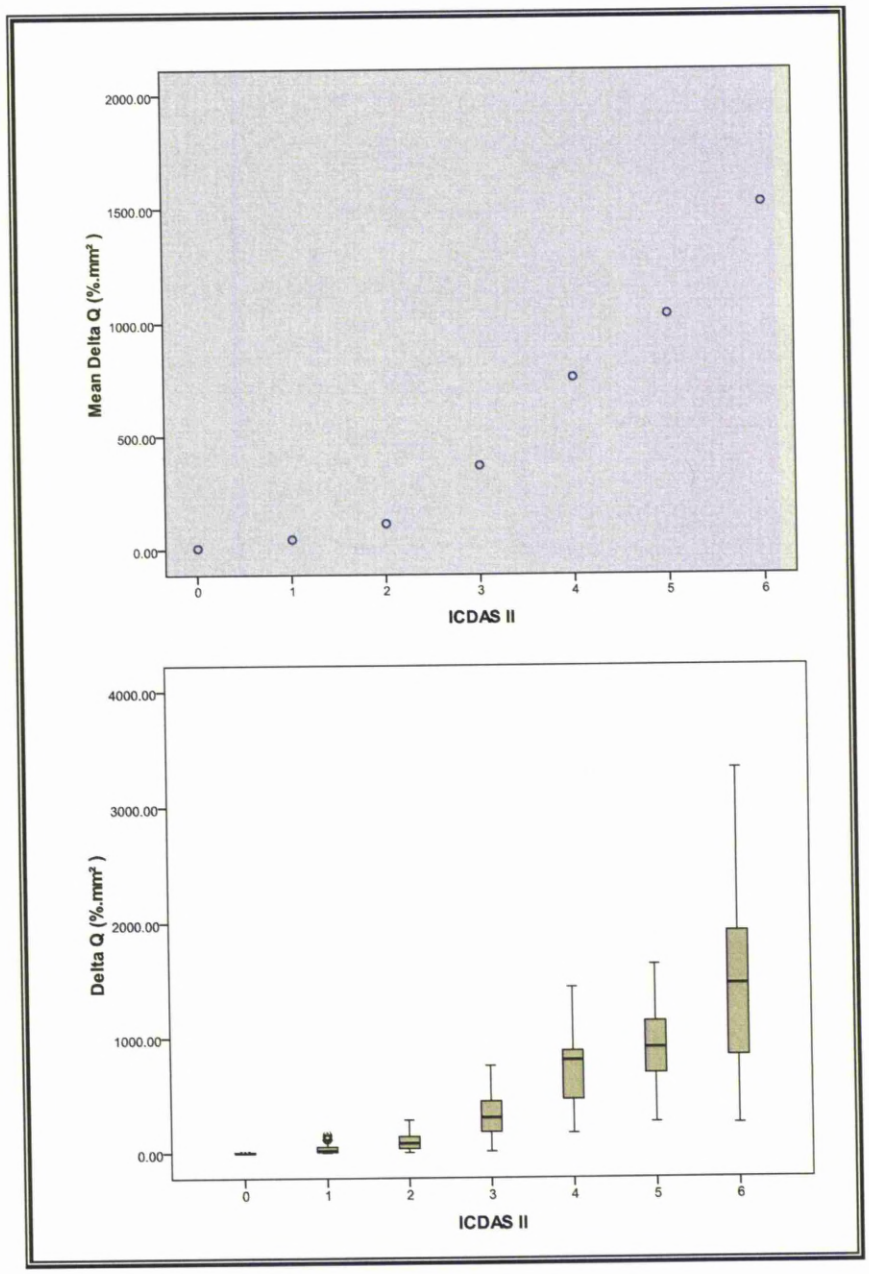


Figure 4.3: Scatter plots of the mean and box plot for ΔQ against ICDAS II for occlusal surfaces.

4.7.1.3 ICDAS II with Delta R-QLF.

The results presented in Figure 4.4 show that red fluorescence level ΔR correlated positively with the ICDAS II visual index. The ICDAS II scores increased linearly with the level of red fluorescence.

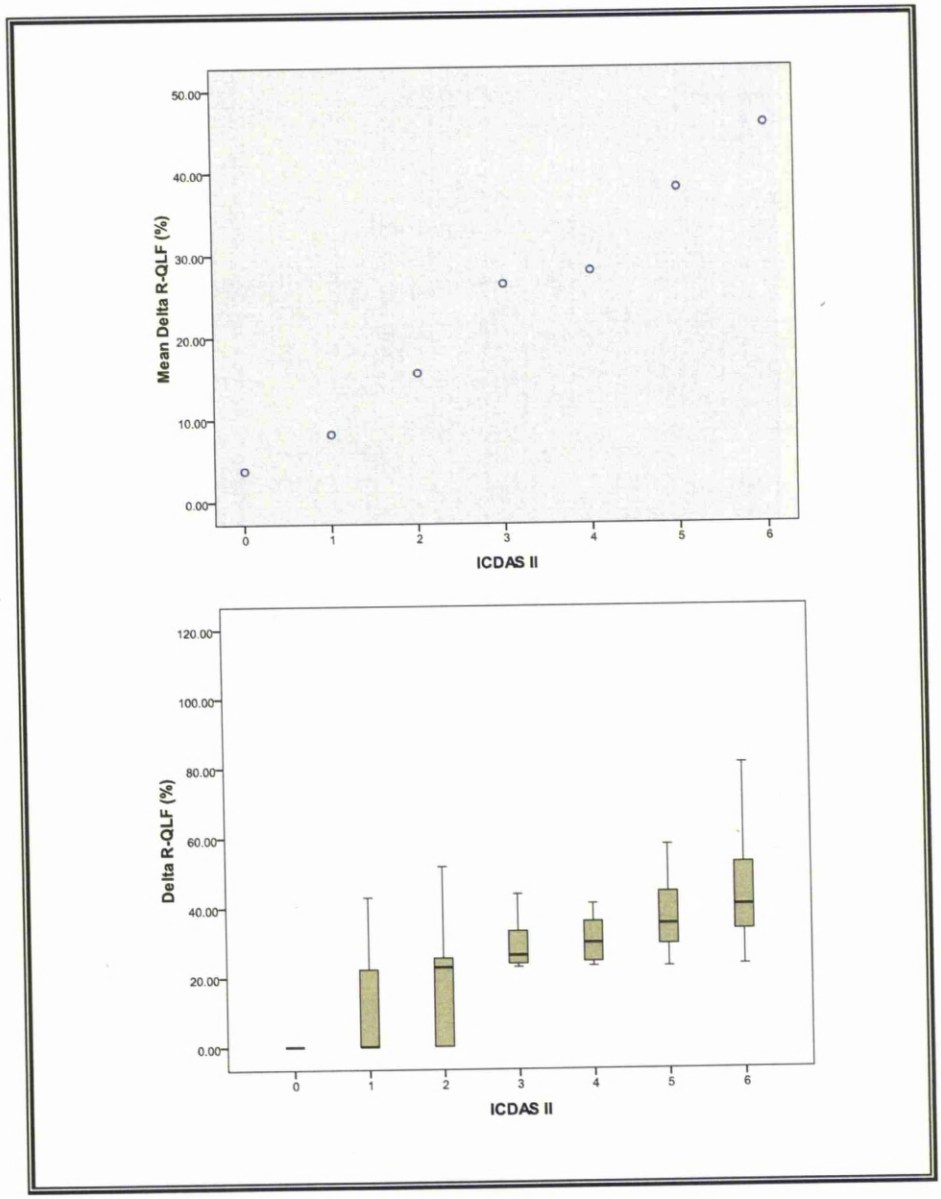


Figure 4.4: Scatter plots of the mean and box plot for ΔR-QLF against ICDAS II for occlusal surfaces.

4.7.1.4 ICDAS II with Delta R-Morita.

The results presented in Figure 4.5 show that the red fluorescence level ΔR-Morita correlated positively with the ICDAS II visual index. The ICDAS II scores increased linearly with the level of red fluorescence on the tooth surface.

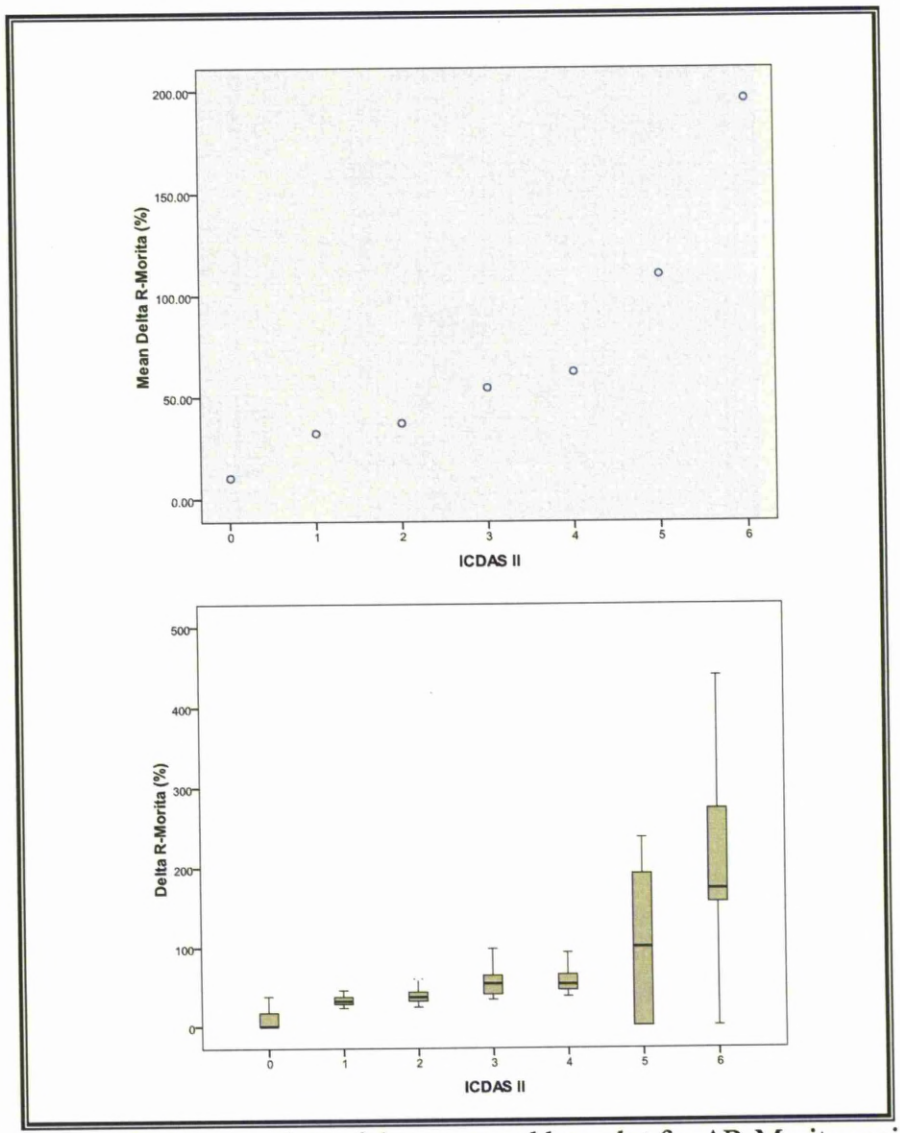


Figure 4.5: Scatter plots of the mean and box plot for ΔR-Morita against ICDAS II for occlusal surfaces.

4.7.1.5 Delta R in QLF and Morita

The images obtained using the Morita camera detected more red fluorescence than QLF camera, example of which are shown in Figure 4.6.

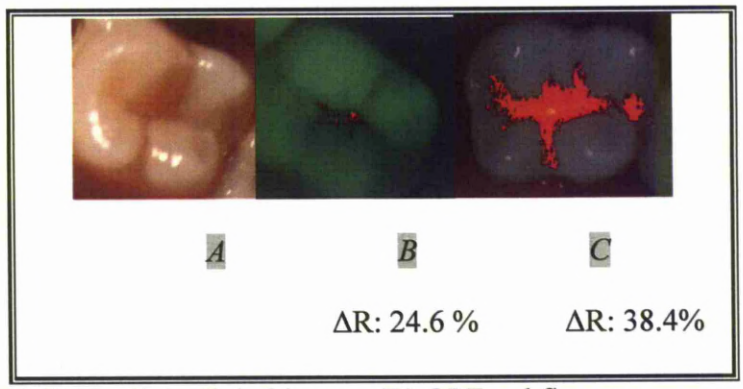


Figure 4.6: (A) White light digital image, (B) QLF red fluorescence analysis image, and (C) Morita red fluorescence analysis image.

4.7.1.6 Histology with delta F

The design of this study allowed teeth which were relatively badly destroyed as a result of dental caries to be extracted and investigated (Figure 4.7). 40 teeth were found to need to be extracted during the course of the study. These teeth had a histological index of 3 and 4 and are presented in this *in vivo* study. Figure 4.8 (A) presents the histology with ΔF values obtained by analysing the occlusal surface of the teeth whereas; Figure 4.8 (B) presents the histology with ΔF values obtained by analysing histological sections after taking their images under QLF conditions. There was a statistically significant difference in ΔF between different histological scores ($p= 0.002$) for ΔF occlusal and ($p= 0.005$) ΔF sections.

Figure 4.7: (A) White light digital image of the occlusal surface, (B)Radiograph image showing the amount of tissue destruction, (C) Histological section of the tooth showing the depth of the lesion, (D) Section under QLF camera showing the amount of mineral loss and fluorescence loss.

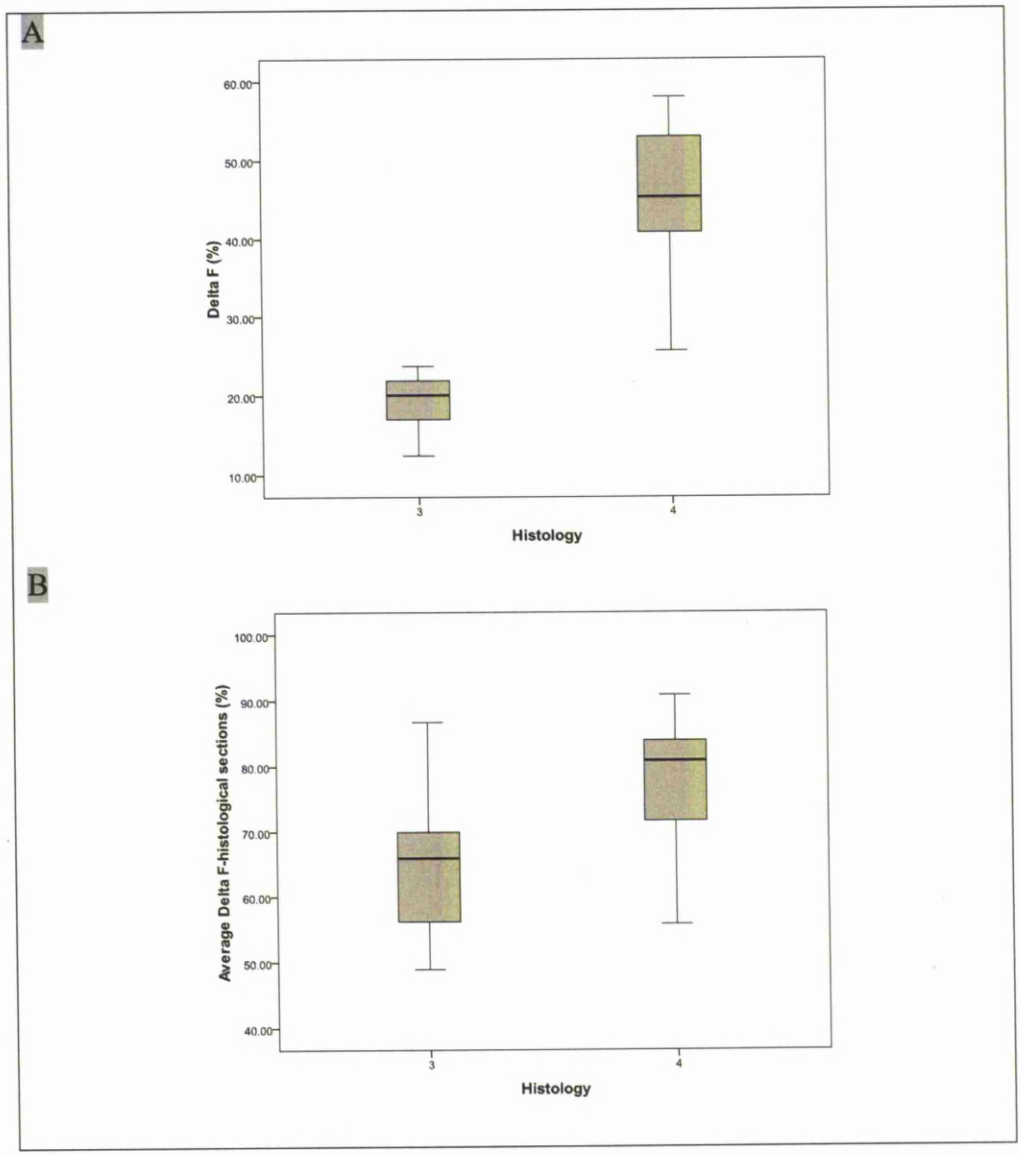


Figure 4.8: (A) box plot for ΔF against histology and (B) ΔF sections against histology for occlusal surfaces.

4.7.1.7 Correlation results from the occlusal surface.

It can be seen from the results presented in Table 4.3 that ΔF correlates well with histology (0.776) and was the best correlation observed. Then, the best correlation was observed between ΔF and ΔQ (0.911) followed by correlation between ΔF and ICDAS II (0.843). In terms of red fluorescence ΔR-Morita data was found to correlate better than ΔR-QLF with ΔF on the occlusal surfaces.

Table 4.3: Correlation between different variables on the occlusal surfaces.

Spearman's rho	ICDAS II	ΔF	ΔQ	ΔR QLF	ΔR Morita	Histology	Radiograph
ICDAS II	1	0.843	0.800	0.613	0.576	0.770	0.756
ΔF	0.843	1	0.911	0.639	0.685	0.776	0.662
ΔQ	0.800	0.911	1	0.720	0.649	0.696	0.627
ΔR(QLF)	0.613	0.639	0.720	1	0.653	0.348	0.523
ΔR(Morita)	0.576	0.685	0.649	0.653	1	0.304	0.477
Histology	0.770	0.776	0.696	0.348	0.304	1	0.610
Radiograph	0.756	0.662	0.627	0.523	0.477	0.610	1

4.7.2 Buccal surface

4.7.2.1 ICDAS II with Delta F

The results presented in Figure 4.9 show that ΔF at the 5% threshold level correlated positively with the ICDAS II visual index. As the ICDAS II values increased the average green fluorescence loss increased. Therefore, as the severity of dental caries increased the fluorescence loss increased on the buccal surfaces of the teeth.

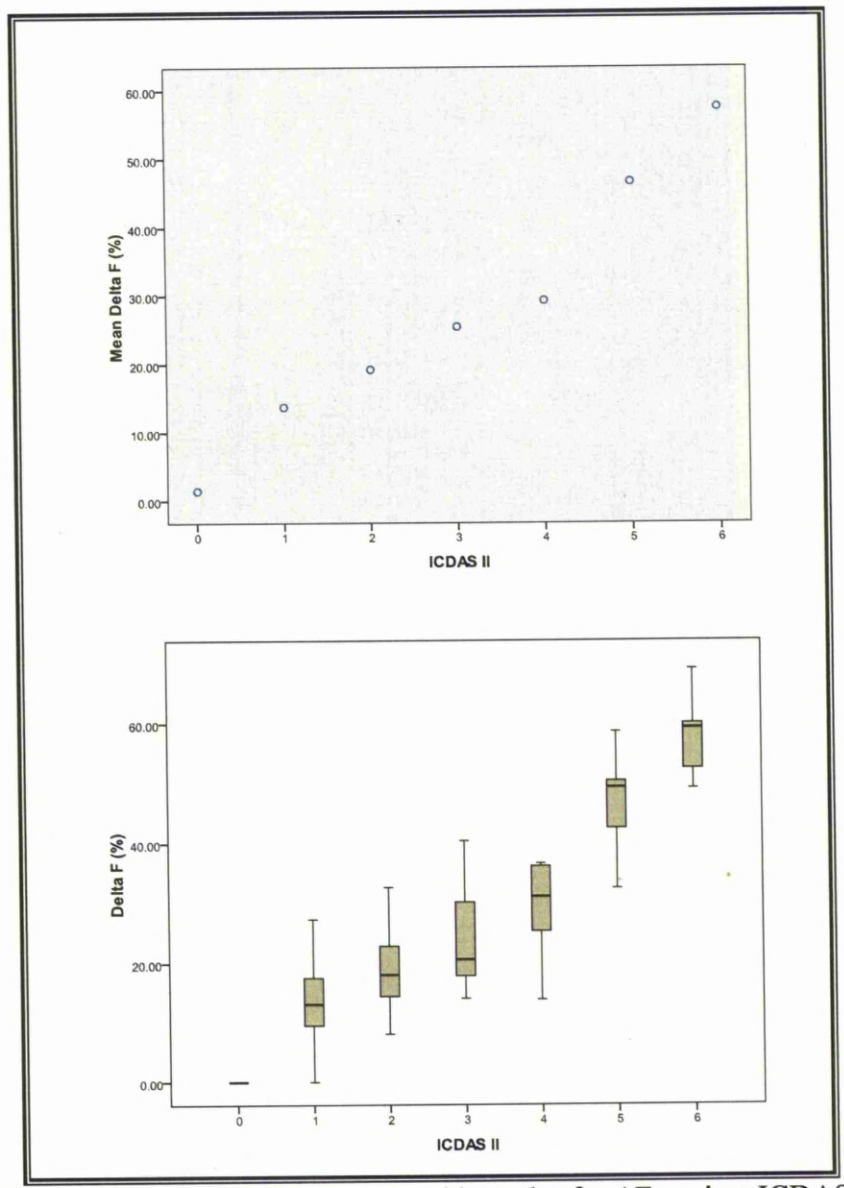


Figure 4.9: Scatter plots of the mean and box plot for ΔF against ICDAS II for buccal surfaces.

4.7.2.2 ICDAS II with Delta Q

The results presented in Figure 4.10 show that ΔQ (area×ΔF) correlated positively with the ICDAS II visual index.

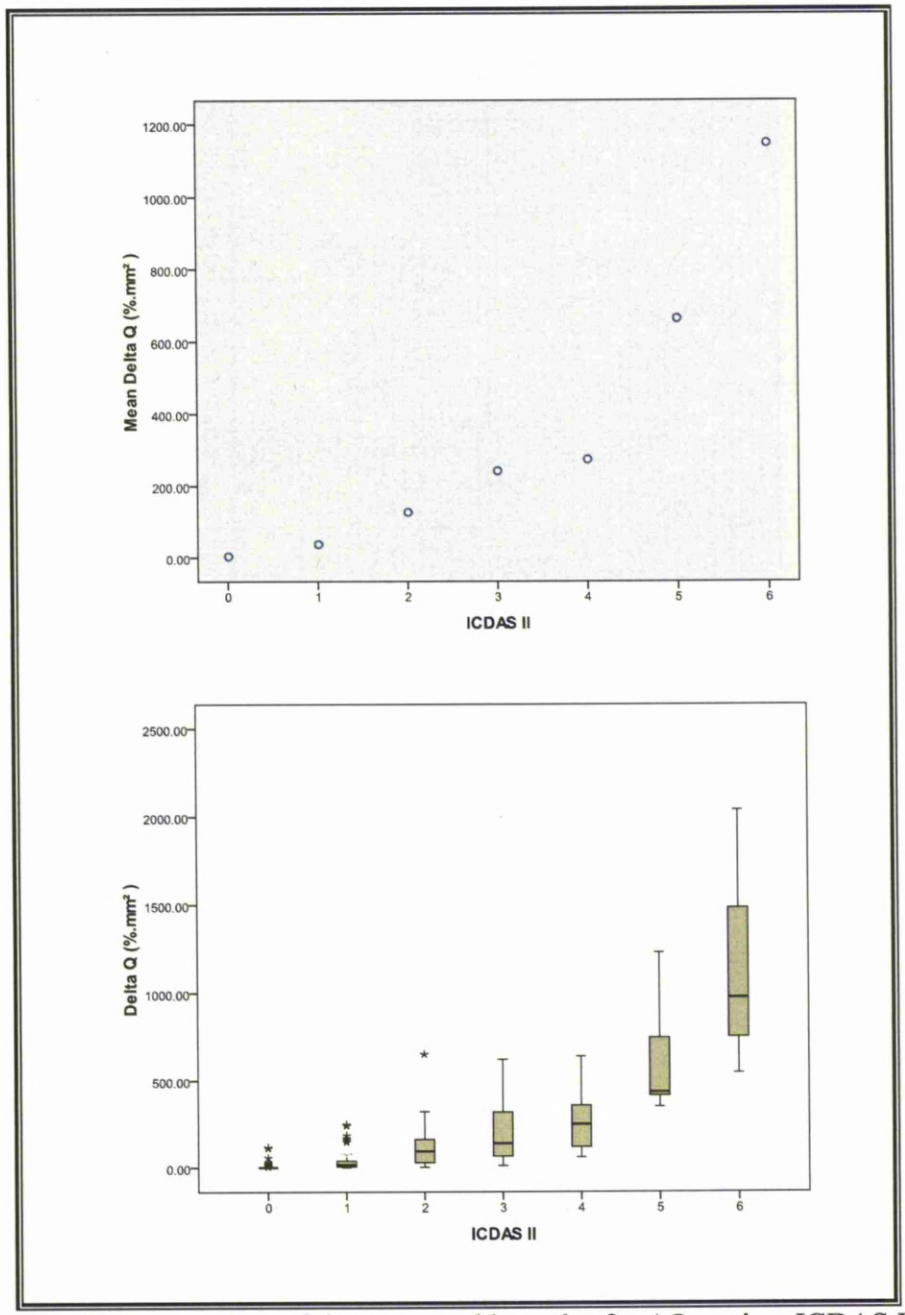


Figure 4.10: Scatter plots of the mean and box plot for ΔQ against ICDAS II for buccal surfaces.

4.7.2.3 ICDAS II with Delta R-QLF

The results presented in Figure 4.11 show that the red fluorescence level, ΔR-QLF correlated positively with the ICDAS II visual index in its early stages (ICDAS II scores 0, 1, 2, 3 and 4).

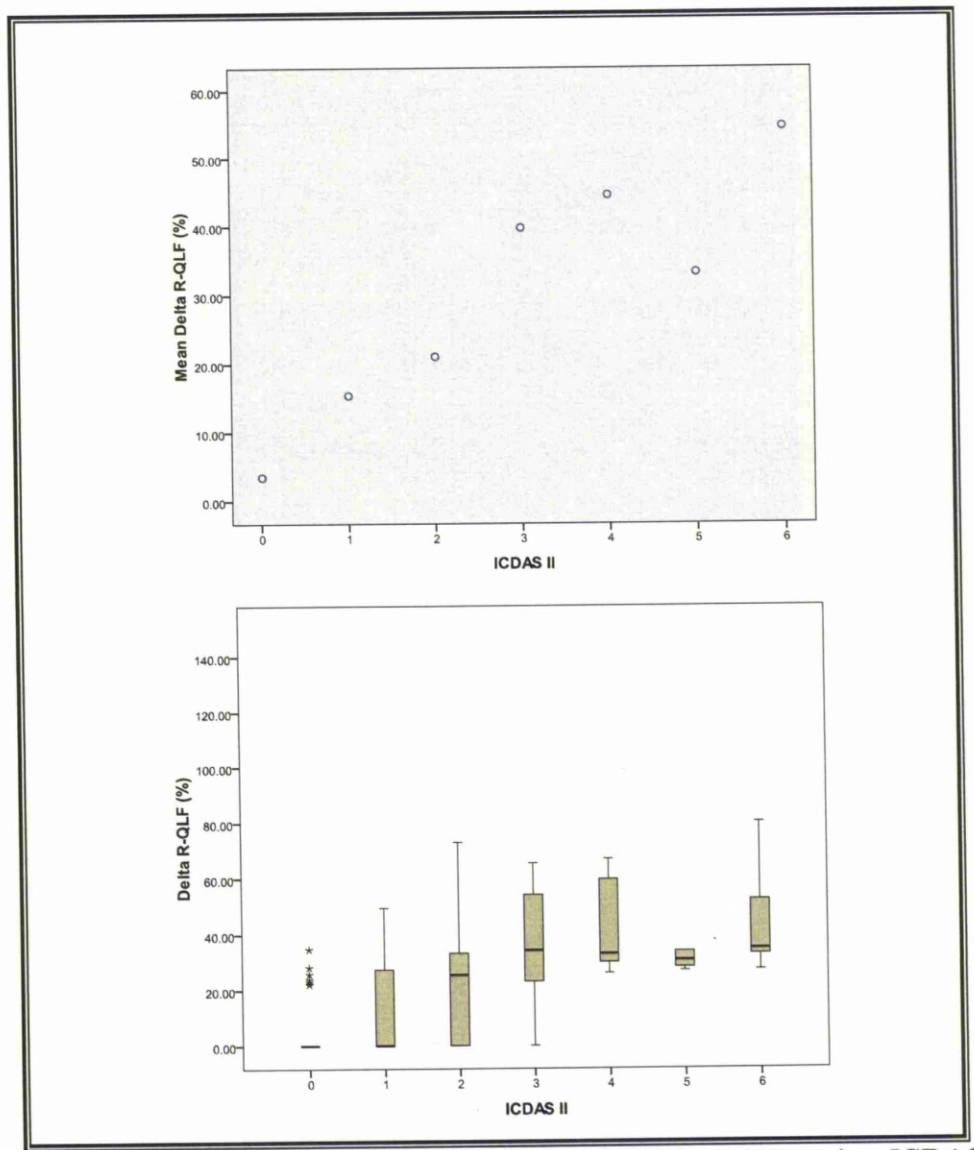


Figure 4.11: Scatter plots of the mean and box plot for ΔR-QLF against ICDAS II for buccal surfaces.

4.7.2.4 ICDAS II with Delta R-Morita

The results presented in Figure 4.12 show that red fluorescence level, ΔR-Morita correlated positively with the ICDAS II visual index in its early stages (ICDAS II scores 0, 1, 2, 3 and 4). It behaved in a similar way to ΔR-QLF but as shown from the values in this Figure the Morita camera images detected greater red fluorescence than QLF.

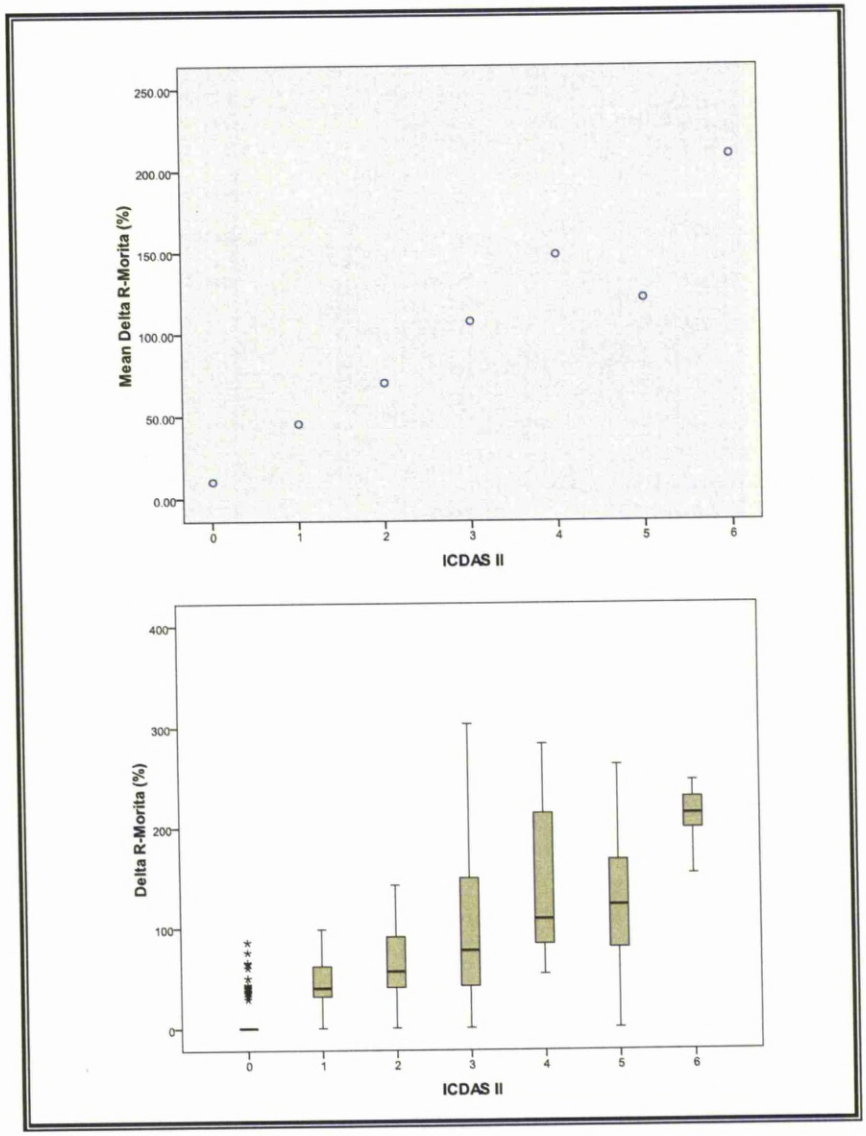


Figure 4.12: Scatter plots of the mean and box plot for ΔR-Morita against ICDAS II for buccal surfaces.

4.7.2.5 Correlation results from the buccal surface

It can be seen from the results presented in Table 4.4 that the best correlation was observed between ΔF with ΔQ (0.959) then ΔF with ICDASII (0.846). In terms of red fluorescence ΔR-Morita correlated better than ΔR-QLF with ΔF on the buccal surface.

Table 4.4: Correlation between different variables on buccal surfaces.

Spearman's rho	ICDAS II	ΔF	ΔQ	ΔR QLF	ΔR Morita	Radiograph
ICDAS II	1	0.846	0.870	0.587	0.694	0.615
ΔF	0.846	1	0.959	0.591	0.751	0.481
ΔQ	0.870	0.959	1	0.574	0.737	0.529
ΔR(QLF)	0.587	0.591	0.574	1	0.654	0.391
ΔR(Morita)	0.694	0.751	0.737	0.654	1	0.381
Radiograph	0.615	0.481	0.529	0.391	0.381	1

4.7.3 Lingual surface

4.7.3.1 ICDAS II with Delta F

The results presented in Figure 4.13 show that ΔF at the 5% threshold level correlated positively with the ICDAS II visual index, as the ICDAS II increased the average green fluorescence loss increased on the lingual surfaces of the teeth.

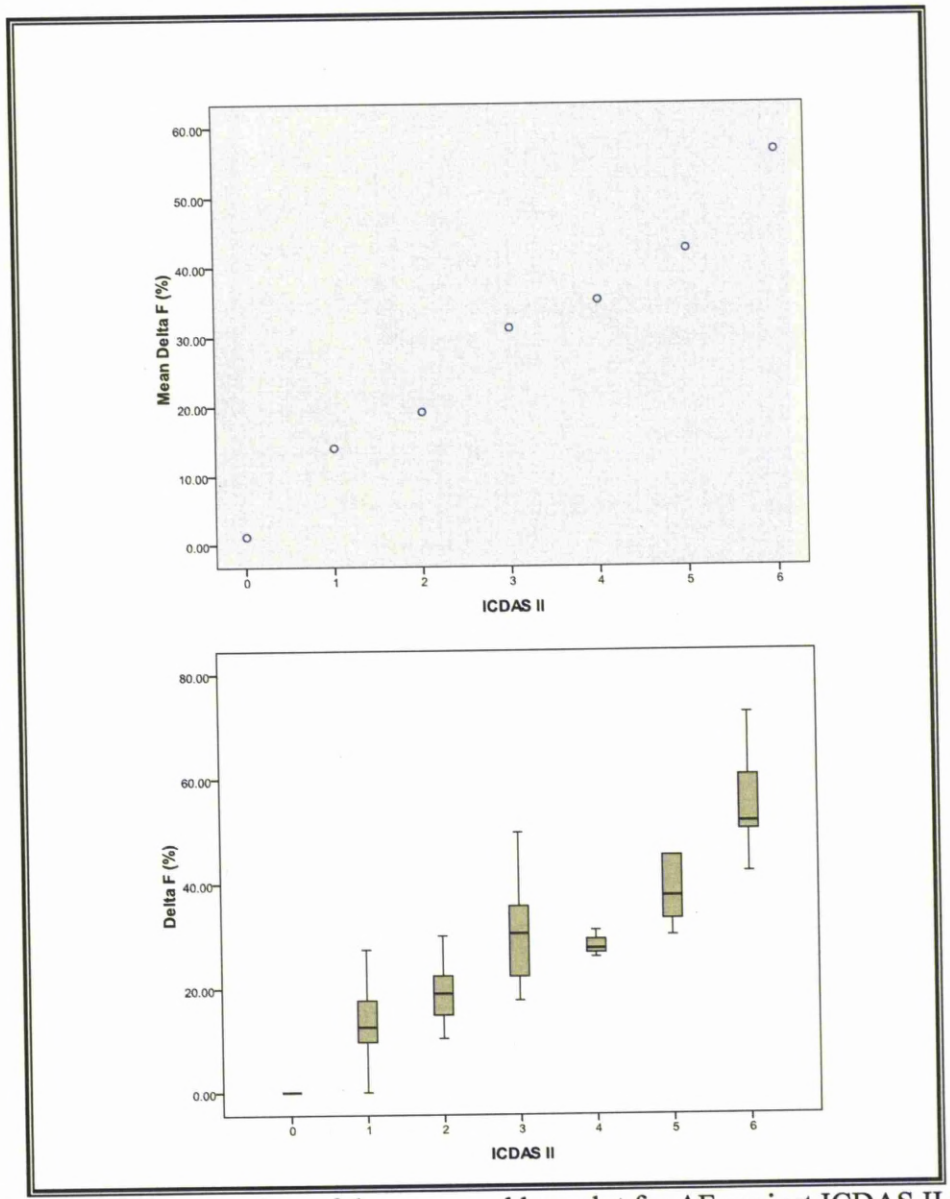


Figure 4.13: Scatter plots of the mean and box plot for ΔF against ICDAS II for lingual surfaces.

4.7.3.2 ICDAS II with Delta Q

The results presented in Figure 4.14 show that ΔQ (area×ΔF) correlated positively with the ICDAS II visual index.

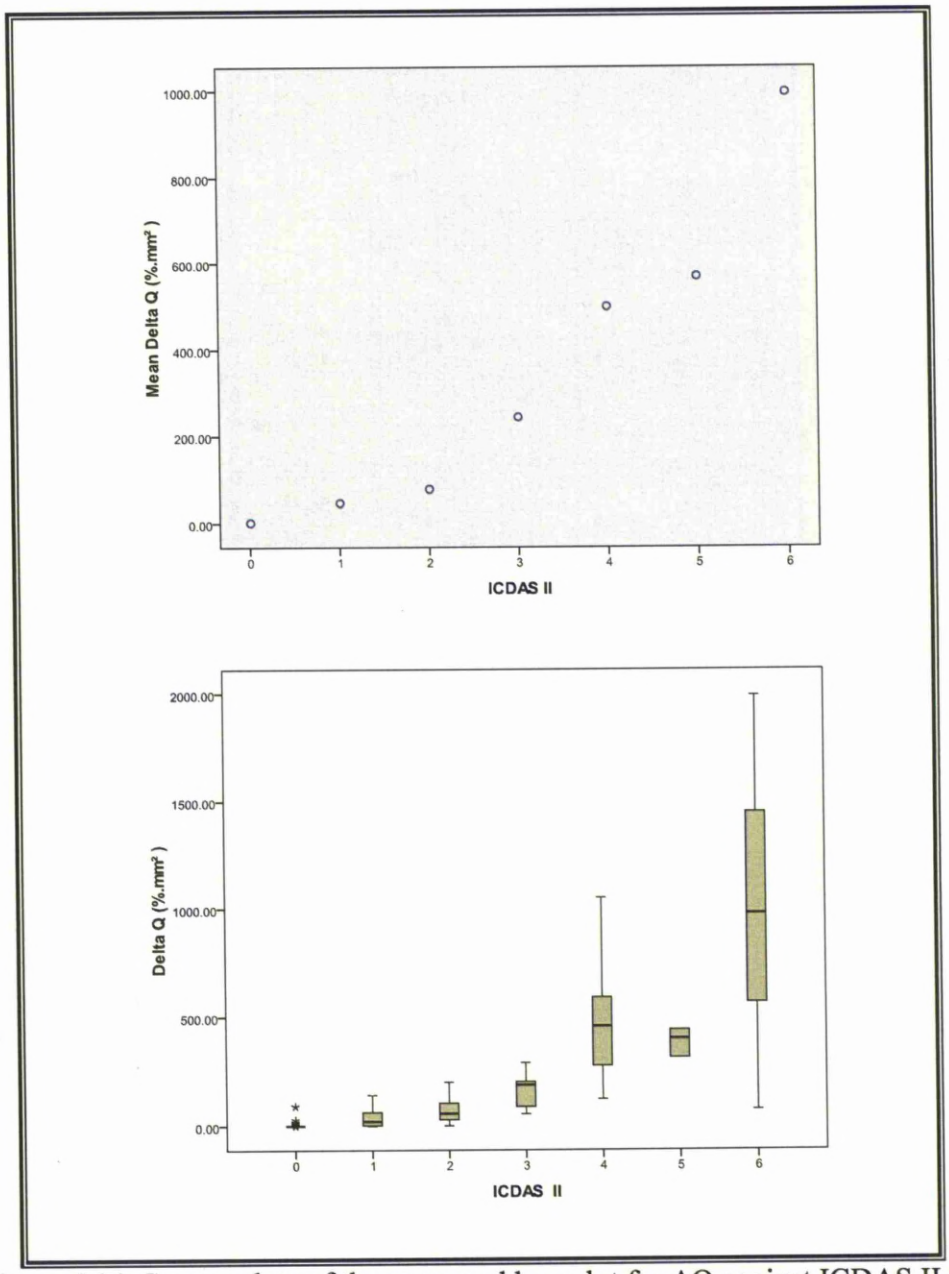


Figure 4.14: Scatter plots of the mean and box plot for ΔQ against ICDAS II for lingual surfaces.

4.7.3.3 ICDAS II with Delta R-QLF

The results presented in Figure 4.15 show that the red fluorescence level, ΔR correlated positively with the ICDAS II visual index in its early stages (ICDAS II scores 0, 1, 2, 3, and 4).

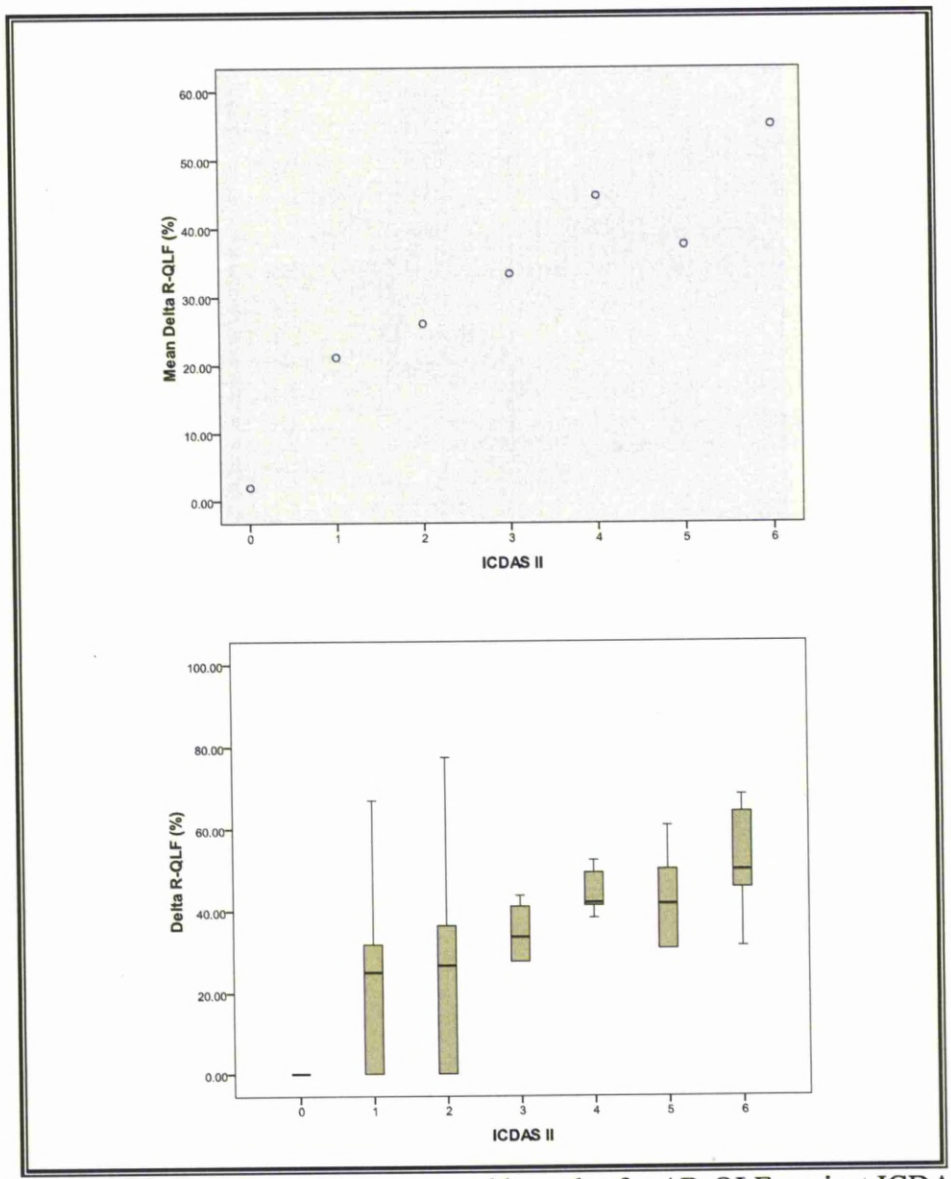


Figure 4.15: Scatter plots of the mean and box plot for ΔR-QLF against ICDAS II for lingual surfaces.

4.7.3.4 ICDAS II with Delta R-Morita

The results presented in Figure 4.16 show that the red fluorescence level ΔR-Morita correlated positively with the ICDAS II visual index. It behaved in a similar

way to ΔR (QLF) but as shown in this Figure the Morita camera detected more red fluorescence than QLF.

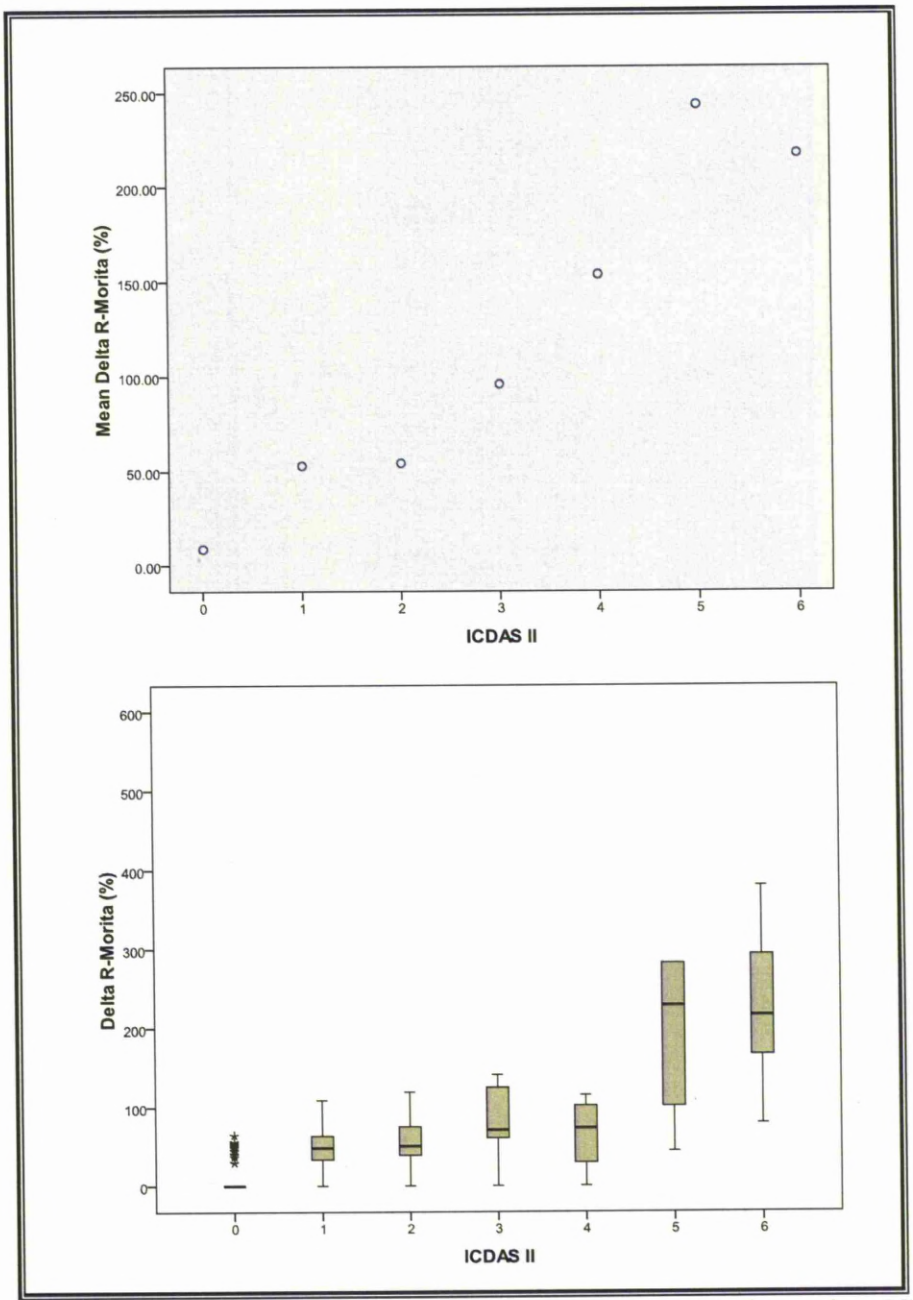


Figure 4.16: Scatter plots of the mean and box plot for ΔR-Morita against ICDAS II for lingual surfaces.

4.7.3.5 Correlation results from the lingual surface

It can be seen from the results presented in Table 4.5 that the best correlation was seen between ΔF and ΔQ (0.977) followed by ΔF with the ICDAS II (0.907). In terms of red fluorescence ΔR-Morita correlated better than ΔR-QLF with ΔF on the lingual surface.

Table 4.5: Correlation between different variables on lingual surfaces.

Spearman's rho	ICDAS II	ΔF	ΔQ	ΔR QLF	ΔR Morita	Radiograph
ICDAS II	1	0.907	0.897	0.668	0.703	0.606
ΔF	0.907	1	0.977	0.633	0.762	0.514
ΔQ	0.897	0.977	1	0.630	0.751	0.522
ΔR(QLF)	0.668	0.633	0.630	1	0.677	0.409
ΔR(Morita)	0.703	0.762	0.751	0.677	1	0.437
Radiograph	0.606	0.514	0.522	0.409	0.437	1

4.8 QLF Clinical Index

The mean, median values and confidence intervals for each QLF parameter and corresponding ICDAS II scores are calculated and the index derived and developed for QLF *in vivo* Table 4.6.

Table 4.6: The QLF *in vivo* index.

QLF Index		ICDAS II CLASSIFICATION		QLF CLASSIFICATION SYSTEM	
				Index	
Code	**Description/ Action**	**Code**	**Description**	**ΔF (%)**	**ΔR (%)**
QLF 0	No dark spots/ no treatment.	0	Sound	-0.5-12	0-20
QLF 1	First visual change in green fluorescence/ preventive treatment and monitoring.	1	Brown/white first visual change in enamel.	12.5-18	21-35
QLF 2	Distinct Visual change in green fluorescence and or appearance of orange red fluorescence/ preventive and or simple conservative restorative treatment.	2	Brown/white distinct visual change in enamel.	18.5-26	36-60
QLF 3	Distinct dark spots and appearance of orange red fluorescence / classic restorative treatment.	3	Localised enamel break-down.	26.5-32	61-88
		4	Underlying shadow.	32.5-38	89-95
QLF 4	Extensive fluorescence loss and dark spots with orange red fluorescence/ Invasive restorative treatment.	5	Distinct cavity.	38.5-48	96-110
		6	Extensive cavity.	>48.5	>110

4.9 QLF Index of choice.

By merging the most appropriate results of QLF parameters obtained and developed from the *in vitro* and *in vivo* studies, the QLF index of choice was produced as shown in Table 4.7.

Table 4.7: The QLF index.

QLF Index		ICDAS II CLASSIFICATION		QLF CLASSIFICATION SYSTEM		
				Index		
Code	**Description/ Action**	**Code**	**Description**	**ΔF (%)**	**ΔQ (%.mm²)**	**ΔR (%)**
QLF 0	No dark spots/ no treatment.	0	Sound	-0.5-12	0-30	0-20
QLF 1	First visual change in green fluorescence/ preventive treatment and monitoring.	1	Brown/white first visual change in enamel.	12.5-18	31-50	21-35
QLF 2	Distinct Visual change in green fluorescence and or appearance of orange red fluorescence/ preventive and or simple conservative restorative treatment.	2	Brown/white distinct visual change in enamel	18.5-26	51-90	36-60
QLF 3	Distinct dark spots and appearance of orange red fluorescence / classic restorative treatment.	3	Localised enamel break-down.	26.5-32	91-280	61-88
		4	Underlying shadow.	32.5-38	281-500	89-95
QLF 4	Extensive fluorescence loss and dark spots with orange red fluorescence/ Invasive restorative treatment.	5	Distinct cavity.	38.5-48	501-700	96-110
		6	Extensive cavity.	>48.5	>700	>110

4.10 Discussion

Caries diagnosis is an important part of the dentist's daily work which can be considered as a three-step procedure: finding the lesion, followed by an assessment of the severity of the lesion, and subsequently followed by an assessment of the activity of the lesion (Ekstrand et al., 2001).

The main objective of this study was to determine whether QLF parameters could be used as an interpretive index in clinical practice especially for occlusal caries. The validity of caries lesion quantification with QLF, a technique primarily developed for smooth surface caries, has been the topic of many studies (Al-Khateeb et al., 1997; Emami et al., 1996; Hall et al., 1997; Lagerweij et al., 1999). For clinical application, the technique should be useful for all stages of lesion progression but with a greater priority given to the early stages which is the most difficult phases to diagnose. The analysis of red fluorescence in a lesion combined with green fluorescence loss has been considered a possible solution to expand the use of QLF to lesions of all depths. To date it has not been possible to interpret QLF parameters in terms of providing useful information in clear, simple and understandable forms to aid clinical decision making.

For use in general dental practice it is necessary to link the QLF outcome measures to clinically relevant measures, i.e. lesion depth in relation to tissue type. In this *in vivo* study clinical caries scores obtained using ICDAS II criteria were chosen to compare QLF outcomes with histology (the parameter of choice *in vitro*). The ICDAS II system measures the surface changes and potential histological depth of

carious lesions by relying on surface characteristics developed by Ekstrand and co-workers (Ekstrand et al., 1995). The ICDAS II criteria incorporate concepts from the research conducted (Ekstrand et al., 1997). These systems indicate that measurement of non-cavitated carious lesions in enamel or dentine can be based on visual topography at the surface level. For the formerly stated reasons ICDAS II was chosen in this study.

In this study ICDAS II visual examination criteria was used to develop a visual code system (VCS) previously described in this thesis (*in vitro* study), but in this *in vivo* study, only 3 digits number representing the (O, B & L) surfaces produced. It gave the dentist quick general information about the presence/absence of dental caries and/or severity of decay.

The quality of the white light digital images taken was not as good as those taken in the *in vitro* study and this is due to the holding of the relatively heavy camera with one hand and supporting the check/lip retractor and the intra oral mirror in the patients' mouth with the other hand. This problem can easily be overcome if a dental nurse or other person could be available to assist and therefore is unlikely to be problematic in most clinical situations.

ICDAS II scores correlated well with histological gold standards on the occlusal surface (0.770). ICDAS II correlated best on all surfaces with ΔF (0.843 on the occlusal surface, 0.846 on the buccal and 0.907 on the lingual surfaces) followed by ΔQ (0.800 on the occlusal. 0.870 on the buccal and 0.897 on the lingual surfaces).

Intra-examiner agreement could not be assessed in this study since it was performed on each patient on a single visit; it was not therefore possible to repeat this measure. This could be a limitation; however the intra-examiner reproducibility in the *in vitro* study involving the same examiner was very good. This limitation is thought to be minimal when considering that the performance obtained *in vitro* tends to be similar to *in vivo* results (Reis et al., 2006).

On the other hand, the ICDAS II correlated less well with radiographs (0.756 on occlusal, 0.615 on buccal and 0.606 on the lingual surfaces) and radiographs correlation with histology on occlusal surface (0.61). This is similar to that reported in other studies, 0.54 (Wenzel and Fejerskov, 1992) and 0.77 (Ekstrand et al., 1997). These results demonstrated that this imaging modality performs poorly as a method to detect occlusal caries.

ΔR-QLF and ΔR-Morita correlated less well on all surfaces with ICDAS II (from 0.576 to 0.703) and with ΔF (0.591 to 0.762). Hence, red fluorescence should be used as a secondary measure only, to indicate bacterial infection in advanced lesion. Sometimes as previously mentioned, it was not always possible to subject the patient to prophylaxis due to the presence of a painful tooth in the mouth. This resulted in more red fluorescence, even in cases where lesions were restricted to enamel, as a result of plaque accumulation on those teeth and/or possibly food remnants as the subjects had avoided brushing the teeth next to the affected area. There was a deviation noted in ΔR-QLF and ΔR-Morita values only for ICDAS II score 5 and this can be easily explained, since there were only seven teeth that fell

in this category on the buccal and lingual surfaces. The images obtained using the Morita camera detected more red fluorescence than those taken with QLF camera. This is thought to be due to the current QLF settings which have been developed for optimal visualisation of mineral loss rather than appropriate red fluorescence. Different filter systems are required for optimal detection of red fluorescence. These filter systems are currently being improved and incorporated into a new QLF model.

The current QLF software presents the analysis results as a pseudo-colour image where different levels of fluorescence loss have their own distinct colour. Complementary to that, the software presents average and integrated fluorescence loss and lesion area. In a separate analysis step the red fluorescence level can be determined and is presented by a corresponding pseudo-colour image with average red fluorescence level and area.

Results suggest that the most suitable QLF parameter to indicate clinical lesion depth score is the average green fluorescence loss. Utilizing this information indices have been derived for the output parameters of QLF.

Sensitivity and specificity for QLF index with histology were high. For histological score 3, it was 0.89, 0.81 (sensitivity and specificity respectively) and 0.82, 0.85 (sensitivity and specificity respectively) for histological score 4. Intra-examiner reproducibility was excellent (0.842) between the first and the second attempts to score the histological sections with a gap of two weeks between them. Regarding

the use of QLF to detect proximal, results from this study indicated that using the current QLF camera handpiece, it is not possible to detect such lesions and this limitation to the diagnosis of proximal surfaces caries have also been noted in other studies performed recently (Buchalla et al., 2001; Buchalla et al., 2002). The reasons for this may be due to the difficulty in positioning the relatively large camera head around the proximal surfaces on premolars and molars. In addition due to the nature of interproximal surfaces with caries, the bulk of sound tooth tissue was often superimposed on the demineralised areas when viewed from the occlusal marginal ridge area and this makes interpretation difficult. Regarding the use of QLF to detect "hidden" or concealed caries, results showed that QLF can detect some of that type of dental caries but more developments are required if it is to be used in diagnosis. These are areas that warrant further development.

Relating QLF fluorescence loss to clinically relevant lesion depth allows useful monitoring of changes in a lesion over time based on quantitative data. This information may then be used to determine the type of clinical intervention required. It may be particularly useful to determine whether preventive and/or operative therapy is required. It was for this reason that an index was developed *in vivo*. It has been demonstrated in this thesis that the index developed *in vitro, showed* less red fluorescence loss in more advanced stages of dental caries than the red fluorescence loss in the *in vivo* study due to the reasons discussed previously which affect the fluorescence results. For this reason, the red fluorescence index developed *in vivo* will be in the QLF index of choice as well as the other QLF

parameters obtained and developed from the *in vitro* and *in vivo* studies as presented in Table 4.7

Issues arising from the use of QLF in this study include access to some surfaces of teeth especially occlusal surfaces in cases where there are third molars in the upper arch of the patients with a small mouth opening; as well as in patients with shallow buccal vestibules. This again is because of relatively large head of the QLF camera. The depth of field of the QLF camera requires that the intra-oral camera needs to move away from the surface undergoing examination in order to get a sharp image and this is not always possible particularly if the patient has an opposing tooth. This can make it more difficult to obtain an excellent quality image and necessitates more time to adjust the camera. These problems could be minimised by a reduction in size of the head of the camera. Finally, the breath of the patients sometimes clouds the mirror and in turn affects the quality of the image obtained. To facilitate and speed up the method in clinical sittings a mirror with air spray tip should be included in the intra oral piece in future designs of this equipment.

4.11 Conclusion

From the results presented in this study, it can be concluded that QLF parameters ΔF, ΔQ and ΔR are useful for the early detection and quantification of mineral loss. In terms of red fluorescence, the Morita camera detected more red fluorescence and expressed the bacterial activity on and/ or within the tooth more effectively than QLF. Red fluorescence correlated well with more advanced lesions and could be

useful as a secondary factor in caries analysis. QLF proved most useful for classifying lesions using ΔF and may aid clinical decision making.

It must be remembered that information from QLF assessment and analysis alone should not form the basis of a clinical decision. QLF is a valuable addition as a diagnostic tool and aid. The work described in this study validated the use of QLF *in vivo* for detecting demineralisation on occlusal, buccal and lingual surfaces. Additionally, an interpretative index was developed which indicates the degree of dental caries involvement and how related this to histology on the occlusal surfaces.

QLF has the potential to play a vital role in the future as caries management moves further towards prevention based on early detection. QLF is non invasive and teeth can be examined repeatedly with no harm to patients or the dental team. It has been found that patients are very interested to view the images of their teeth produced by this device. It is extremely useful to show patients the areas which show early signs of demineralisation that they should concentrate on, thus it is anticipated that this may increase the patients' motivation as well. Therefore, it is envisaged that QLF will have the potential for wider application in clinical dentistry than just early caries diagnosis. It may subsequently, aid in prevention of demineralisation and/ or promotion of remineralisation by preventive measures.

CHAPTER 5

THE *IN VIVO* STUDY 2

Quantitative light-induced fluorescence (QLF): A potential tool for early occlusal dental caries detection and supporting decision making *in vivo*.

5.1 Introduction

An increasing proportion of the total caries burden is found in fissures (Truin et al., 1993). This is perhaps a result of the superficial remineralisation potential of low concentrations of fluoride over extended periods. Recent changes in lesion morphology mean that occlusal dentinal caries can be present under a fissure which appears intact to the naked eye. Detection of occlusal caries is recognised to be difficult (Kidd et al., 1993b; Lussi, 1991; Pitts, 1997).

Repair of the consequences of dental caries is costly in terms of time, resources, and oral health. The prevention of demineralisation, the endorsement of remineralisation of early stages of dental caries and the early restorative intervention in the case of incipient caries are therefore the main aims of contemporary dentistry. However, these goals can only be achieved if caries is detected at an early stage with a tool capable of helping the clinician to reach a more objective clinical decision before the need for more invasive restorative intervention. Although regular visits to the dental surgery are recommended, diagnostic methods in common use during these visits have limitations. They only have the capability to detect caries at a relatively advanced stage, and cannot quantitatively assess the mineral changes occurring over time (Ekstrand et al., 1995; Wenzel et al., 1991b). The problem is confounded more by the poor performance of radiographs to detect early enamel caries (Thomas et al., 2001). Classically occlusal caries is only noticeable on the radiograph when they are in

dentine and may be more severe than what it is indicated on the radiograph (Ekstrand et al., 1995).

QLF measures the percentage of fluorescence change of demineralised enamel with respect to surrounding sound enamel, and relates it directly to the amount of mineral lost during demineralisation. In early studies the ability of QLF to detect and quantify caries was determined and compared the technique with other destructive methods (Al-Khateeb et al., 1997; Ferreira Zandona et al., 1998b).

Early detection of lesions constitutes a major goal in the attempt to move away from operative to non-operative, preventive treatment, at the same time as aiming to control and manage the development of the disease process throughout life (Pitts, 2004b). Consequently, successful management of occlusl caries lesions requires an accurate and reliable detection and diagnosis of non-cavitated lesions.

5.2 Aim and objectives

5.2.1 Aim

In view of the increasing interest in the development of techniques to supplement the detection accuracy of traditional methods and because of the limited research available in this area, the aim of the study was to evaluate whether QLF parameters were appropriate and useful as an aid to diagnosis and clinical decision making of early occlusal caries by comparing QLF analysis with actual restorative management.

5.2.2 Objectives

The objectives of this study were to determine whether QLF is an appropriate technique to quantify *in vivo* the presence and the extent of mineral loss in cases where the diagnosis is difficult regarding the degree of mineral loss affecting the occlusal surface of the posterior teeth. Finally, the evaluation of QLF as a tool to support and aid in decision making regarding the type of intervention required on the occlusal surface of the posterior teeth.

5.3 Ethical approval

All the necessary documents and forms were completed and the required approval granted (University of Liverpool sponsorship letter, Reference: 000319-Appendix10) then, submitted to Liverpool Adult Local Research Ethics Committee. Ethics approval (Reference number: 08/H1005/50- Appendix 11) and NHS Research and Development approval was obtained (Appendix 12).

5.4 Recruitment

46 subjects attending The Liverpool University Dental Hospital were enrolled in this study. Recruitment was achieved from casual patients attending the routine students' clinics at the restorative departments. Those students were supervised by clinical lecturers.

5.4.1 Subjects information sheet and consent

Each patient received a copy of the subject information sheet (Appendix 13) and they were informed about the anonymisation of their names and teeth prior to initiation of the study and if they were willing to take part, signed the consent form (Appendix 14).

5.4.2 Criteria for selection

5.4.2.1 Inclusion criteria

A subject was eligible for enrolment provided that the following criteria were met:

1. Subject is 18-75 years of age.
2. Subject has read patient information sheet, signed the informed consent prior to initiation of study procedure.
3. Presence of radiograph of the tooth which shows no caries and treatment plan signed by the relevant supervisor at the treatment plan appointment for either a fissure sealant (FS), preventive resin restoration (PRR) or a class I occlusal restoration treatment.
4. Presence of one posterior tooth with suspected occlusal caries confirmed again by the student's supervisor on the same day.

5.4.2.2 Exclusion criteria

A subject could not be enrolled in the study if any of the following criteria were met:

1. Edentulous.
2. Subjects have no posterior tooth with suspected occlusal caries.
3. Concurrent participation in another clinical study or within 30 days of participation.
4. Subject is pregnant (based on oral interview only).

5.4.3 Sample size

Following consultation with a statistician the sample size was calculated to allow detection of a statistically significant difference in delta F (ΔF). The power of the study was set at 80%, and α was set at 0.05. Using these values, the calculation produced a sample size of 23 subjects per group, giving a total of 46 subjects in the study.

5.5 Clinical procedures

The procedure was as follow:

1. The patient was given a written information sheet with a verbal explanation.
2. Written consent was obtained.
3. Non-cavitated and non-restored occlusal surfaces of posterior teeth were classified according to the visual criteria of ICDAS II; all visual decisions were made under standardised conditions using a dental unit equipped with compressed air and evacuation facilities (KaVo Dental, Biberach/Riss, Germany).

4. A White light digital image (WLI) (Nikon D200 SLR Camera, Japan) of the tooth's occlusal surface which has suspected occlusal caries was taken (Figure 5.1-A).
5. A QLF image was obtained using a portable QLF device (QLF/clin) (Inspector Research Systems BV, Amsterdam, The Netherlands) of the tooth's occlusal surface after 5s air drying (Figure 5.1-B).
6. An image was obtained using the Morita Intra oral Camera (J. Morita MFG. CORP., Tokyo, Japan) (MCI) of the tooth's occlusal surface (Figure 5.1-C).
7. An image of the radiograph was obtained while it was on the X-ray viewer (Nikon D200 SLR Camera, Japan) (Figure 5.1-G).
8. The clinical procedure sheet developed by the researcher for this study was completed (Appendix 15). This involved information regarding the tooth number, ICDAS II score for the occlusal surface, radiographic index and details regarding the clinical procedure that was finally undertaken on the tooth.
9. After the student drilled an initial cavity (usual routine procedure) and before a decision was made whether to proceed with caries removal, images were obtained as above (WLI, QLF image MCI) (Figure5.1-F, D and E). The restorative treatment decision determined by the supervising clinician was made independent of any imaging performed.
10. The clinical procedure reflecting the extent of operative intervention undertaken was recorded as a fissure sealant/ preventive resin restoration (FS/ PRR) or a class I occlusal restoration (Rest.).

11. All the images obtained were analysed using the QLF software for white spots (WS) and red fluorescence (RF) blinded to the treatment outcome.

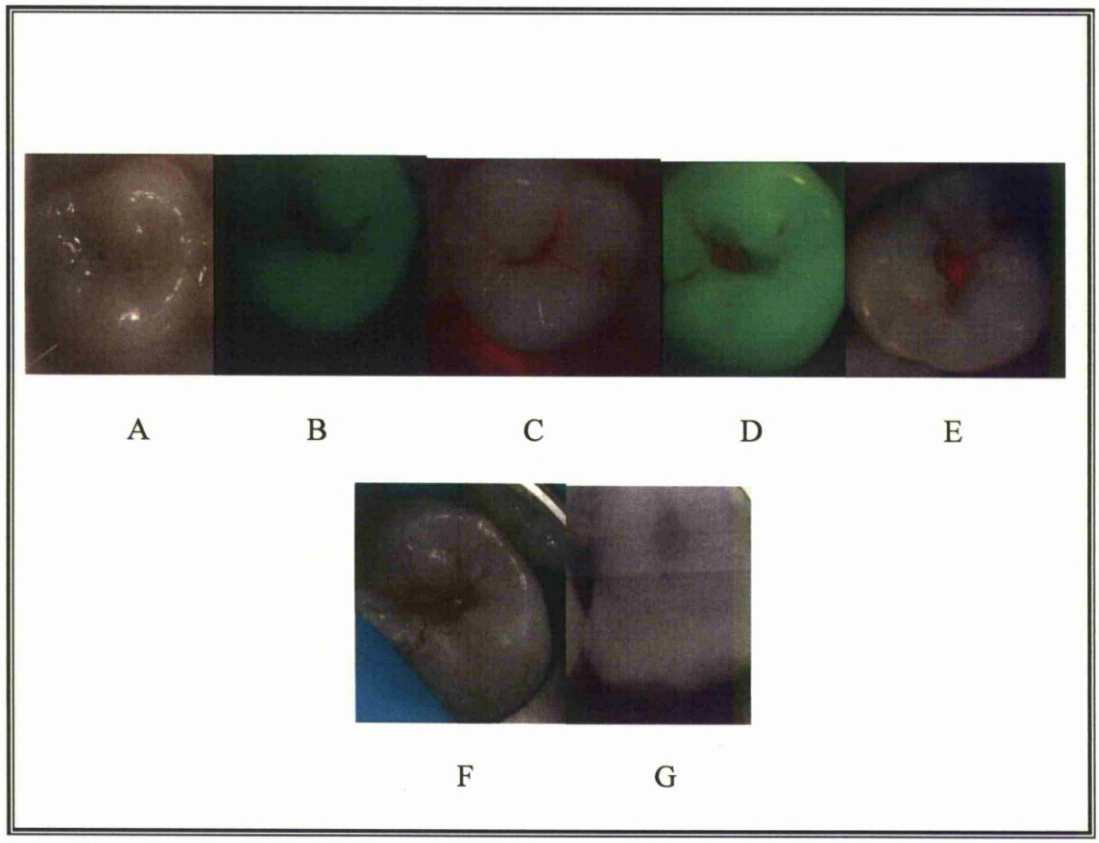


Figure 5.1:(A) White light image of molar with suspected areas, (B) QLF image showing demineralised areas, (C) Morita image showing red fluorescence, (D) QLF image of tooth showing localised areas of decay (dark spot) and red fluorescence, (E) Morita image showing red fluorescence after drilling and the caries removed, (F) White light image after drilling confirming the presence of caries. (G) Radiograph.

5.6 Analysis

5.6.1 QLF analysis

QLF images before and after drilling the tooth were stored on the QLF PC. Each image was analysed by a single examiner for (WS) and (RF) independently by the investigator using QLF software (Inspektor Pro 2.0.0.39,

Inspektor Research System BV, Amsterdam, The Netherlands) without knowing the treatment regimen chosen. A standard analysis technique was employed. The lesion was marked on the screen to ensure analysis of the areas determined as caries. For every lesion, ΔF (%), the area of the lesion (mm^2) and ΔQ (i.e. the product of these two parameters, $\%.mm^2$) were calculated by the software at the 5% QLF threshold. Red fluorescence analysis was conducted in the same way for each lesion; ΔR (%) and red fluorescence area (mm^2) were calculated using the software as shown in Figure 5.2. The ΔQ, Δ F and WS area together with ΔR and RF areas values were recorded. Images of teeth were then reassessed after a two week interval and, if the investigator came to different findings, repeated the analysis until reaching agreement. After completion of the QLF analysis, the data were decoded and entered into SPSS for statistical analysis.

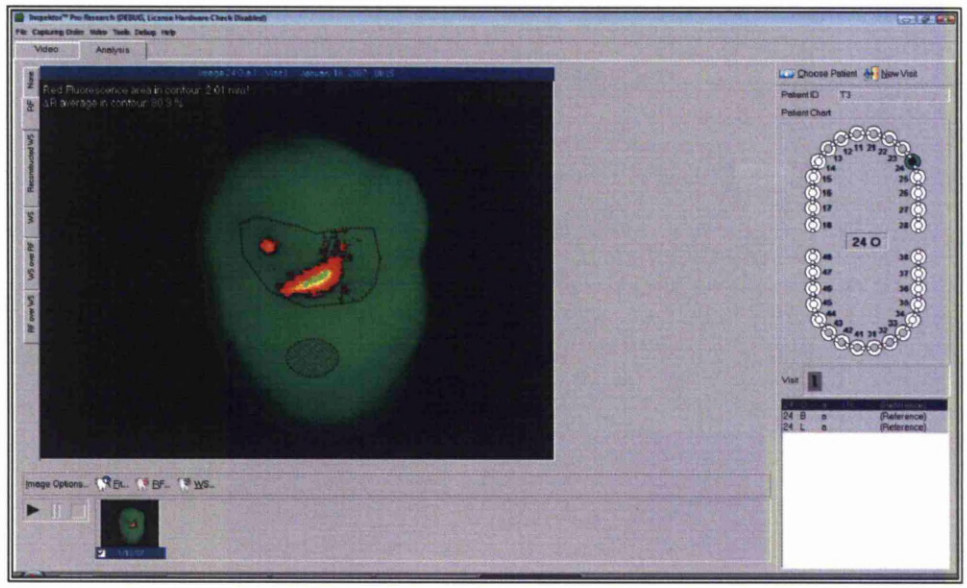


Figure 5.2: Image representing the red fluorescence analysis in QLF software.

5.6.2 Morita analysis

All the Morita images were stored on the same QLF PC. Each image was analysed for RF using QLF software. ΔR (%) and RF area (mm^2) values were recorded; the data were decoded and entered into SPSS for statistical analysis.

5.7 Statistical methods

All the data obtained were analysed statistically by the use of SPSS for Windows software (Version 17.0). Descriptive statistics included mean values, median values, standard deviations and 95% confidence intervals for the parameters ΔF, ΔQ, ΔR and white spot area. Independent samples T-test and paired samples T-test were also used a p-value <0.05 was considered statistically significant.

5.8 Results

5.8.1 Occlusal ΔF at the baseline

Table 5.1: Mean and median for ΔF values in the two treatment groups at baseline.

Treatment	n	Mean	Median
FS or PRR	23	22.60	22.90
Restoration (Simple class I occlusal)	23	28.76	27.60

Independent samples T-test was performed and a statistically significant difference in delta F (ΔF) was found between teeth which were found to have caries and required simple class I occlusal restorations, and those which were caries free and required either a fissure sealant (FS) or preventive resin restoration (PRR) (p=

0.002). Results presented in Table 5.1 show the difference between the mean and the median at the baseline in the two groups.

5.8.2 ΔF in the two treatment groups

Table5.2: Mean and standard deviation of ΔF before and after drilling in the two groups.

ΔF	Mean	Standard Deviation
Before drilling (PRR)	22.60	5.60
After drilling (PRR)	22.11	4.73
Before drilling (Restoration)	28.76	6.06
After drilling (Restoration)	31.95	5.88

The results in Table 5.2 and Figure 5.3 show a reduction in the mean in the PRR group from 22.60 ± 5.60 before drilling the tooth to 22.11 ± 4.73 after the drilling. There was no statistically significant difference found (p= 0.73) in the same group before and after drilling despite the reduction in the mean. On the other hand, in the class I restoration group the mean increased from 28.76 ± 6.06 before drilling to 31.95 ± 5.88 after drilling. Their difference were not statistically significant (p= 0.14).

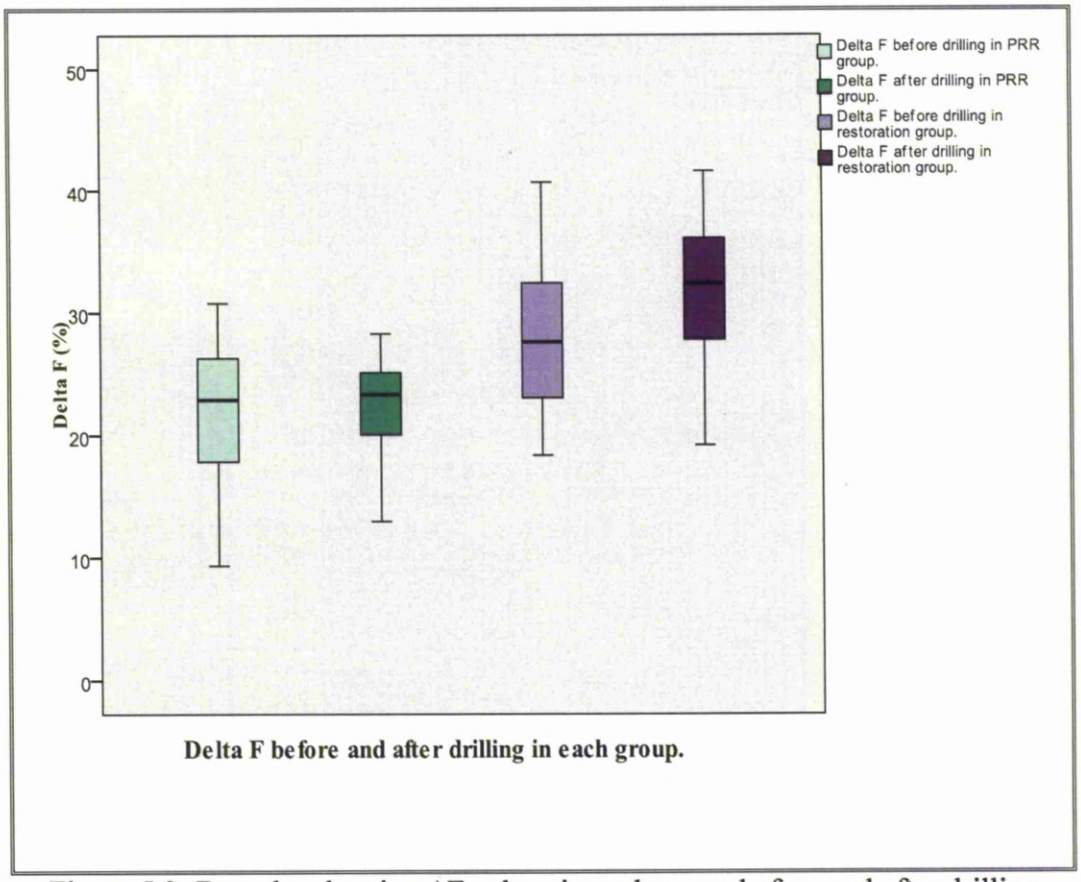


Figure 5.3: Box plot showing ΔF values in each group before and after drilling teeth.

5.8.3 ΔQ in the two treatment groups

Table 5.3: Mean and standard deviation of ΔQ before and after drilling in the two groups.

ΔQ	Mean	Standard Deviation
Before drilling (PRR)	230.49	161.82
After drilling (PRR)	214.31	135.43
Before drilling (Restoration)	348.30	235.94
After drilling (Restoration)	391.73	180.48

Results in Table 5.3 and in Figure 5.4 show that there was a reduction in the mean ΔQ 230.49 ± 161.82 before drilling to 214.31±135.43 after drilling with no statistically significant difference between them (p= 0.62). However, in the class I restorations group the increase in the mean was from 348.30 ± 235.94 to 391.73 ±

180.48 but again with no statistically significant difference between them (p= 0.34).

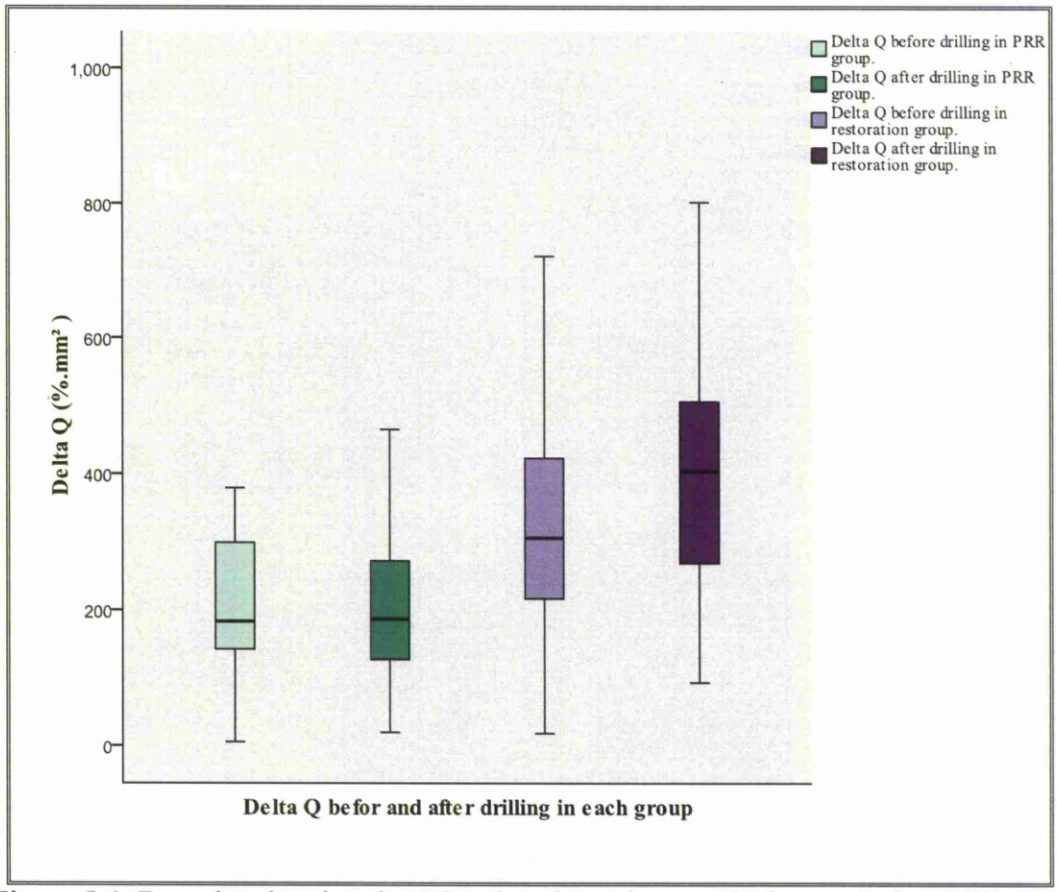


Figure 5.4: Box plot showing the ΔQ values in each group before and after drilling teeth.

Independent samples T-test was performed and a statistically significant difference in delta Q (ΔQ) was found between teeth which are found to have caries and needed simple class I occlusal restoration, and those which are not carious and needed only either FS or PRR (p= 0.012).

5.8.4 ΔR-QLF in the two treatment groups

Table 5.4: Mean ΔR-QLF before and after drilling in the two groups.

ΔR (QLF)	Mean	Standard Deviation
Before drilling (PRR)	26.65	3.00
After drilling (PRR)	28.52	10.01
Before drilling (Restoration)	27.79	7.93
After drilling (Restoration)	31.36	8.44

Results in Table 5.4 and in Figure 5.5 show that the mean ΔR increased from 26.65 ± 3.00 before drilling to 28.52 ± 10.01 after drilling in the FS and the PRR group as well as in the simple class I occlusal restoration group where it increased from 27.79 ± 7.93 to 31.36 ± 8.44. In this parameter there was no statistically significant difference in the FS/ PRR group ($p= 0.32$) but there was a highly statistically significant difference in the simple class I occlusal restoration group ($p= 0.002$).

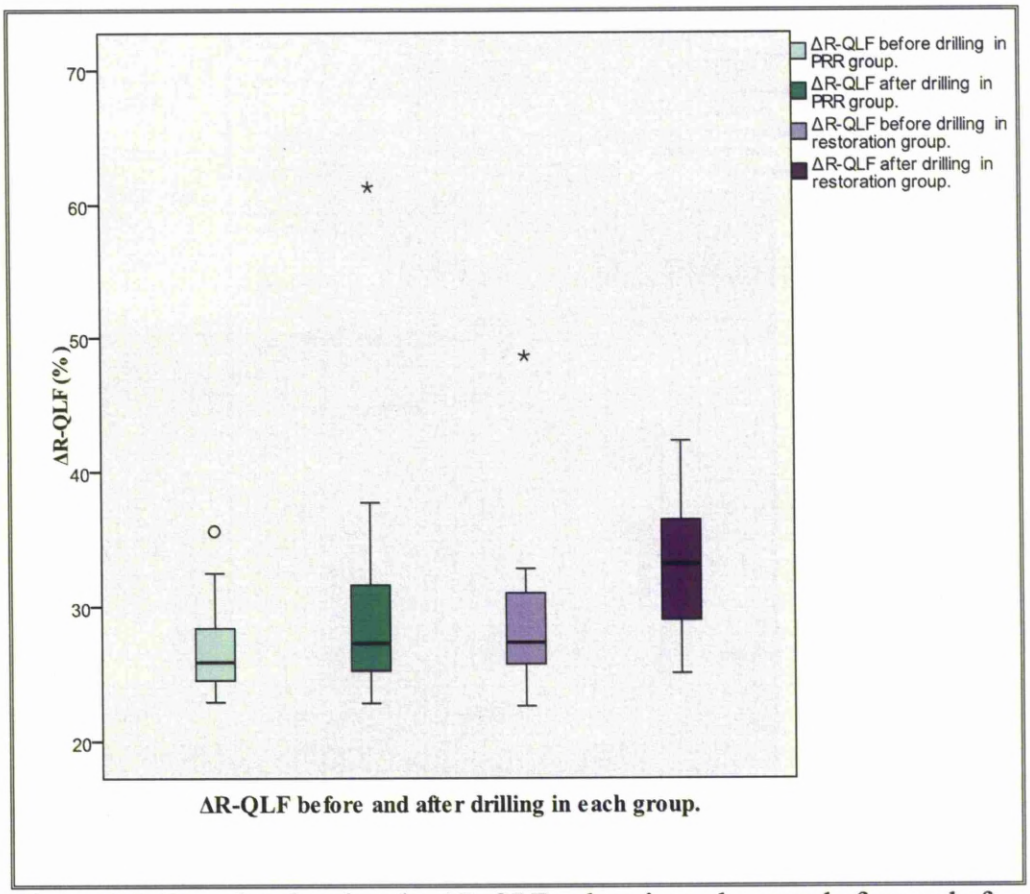


Figure 5.5: Box plot showing the ΔR-QLF values in each group before and after drilling teeth.

Independent samples T-test was performed with no statistically significant difference in ΔR-QLF between teeth which were found to have caries and required simple class I occlusal restoration, and those which were not carious and only needed either FS or PRR ($p= 0.78$).

5.8.5 ΔR-Morita in the two treatment groups

Table 5.5: Mean ΔR-Morita before and after drilling in the two groups.

ΔR (Morita)	Mean	Standard Deviation
Before drilling (PRR)	46.07	8.41
After drilling (PRR)	48.93	12.79
Before drilling (Restoration)	56.61	15.89
After drilling (Restoration)	66.49	16.74

ΔR-Morita gave similar results to those of ΔR-QLF, showing an increase in the mean from 46.07 ± 8.41 to 48.93 ± 12.79 in FS/ PRR group and there was an increase in the mean in the simple restoration group from 56.61 ± 15.89 to 66.49 ± 16.74 as presented in Table 5.5 and in Figure 5.6. There was a statistically significant difference (p= 0.016) between the simple occlusal class I restoration group before and after drilling whereas there was no statistically significant difference in the FS/ PRR group (p= 0.28).

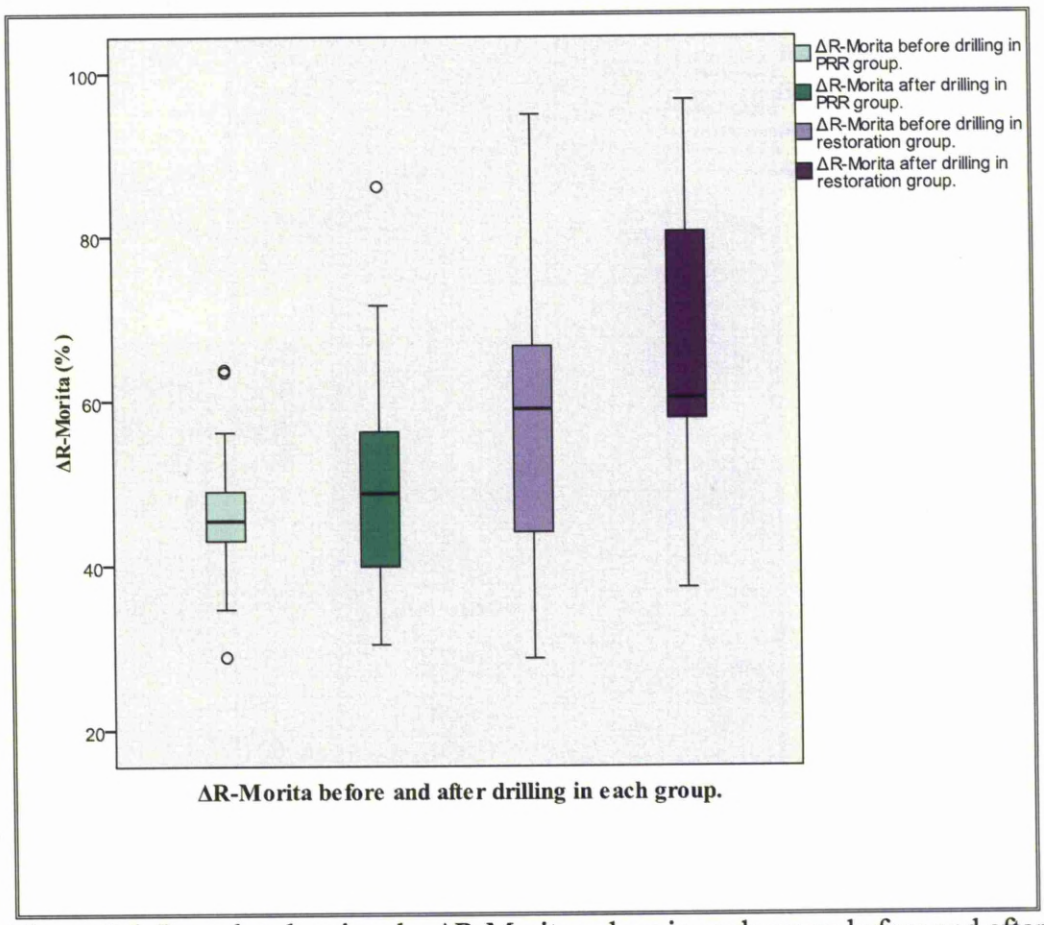


Figure 5.6: Box plot showing the ΔR-Morita values in each group before and after drilling teeth.

Independent samples T-test was performed and a statistically significant difference in ΔR Morita was found between teeth which were found to have caries and needed a simple class I occlusal restorations, and those which were not carious and required only either FS or PRR ($p= 0.009$).

5.8.6 White spot area (WS) in the two treatment groups

Table 5.6: Mean white spot area (WS) before and after drilling in the two groups.

WS	Mean	Standard Deviation
Before drilling (PRR)	9.90	5.54
After drilling (PRR)	8.97	5.03
Before drilling (Restoration)	12.03	5.89
After drilling (Restoration)	12.07	5.01

Results in Table 5.6 and in Figure 5.7 demonstrate that there was a decrease in the mean of WS area in the FS/ PRR group from 9.90 ± 5.54 before drilling to 8.97 ± 5.03 after drilling and there was a slight increase in the simple class I occlusal restoration from 12.03 ± 5.89 to 12.07 ± 5.01 with no statistically significant difference in both groups. Independent samples T-test was performed and no statistically significant difference found (p= 0.215).

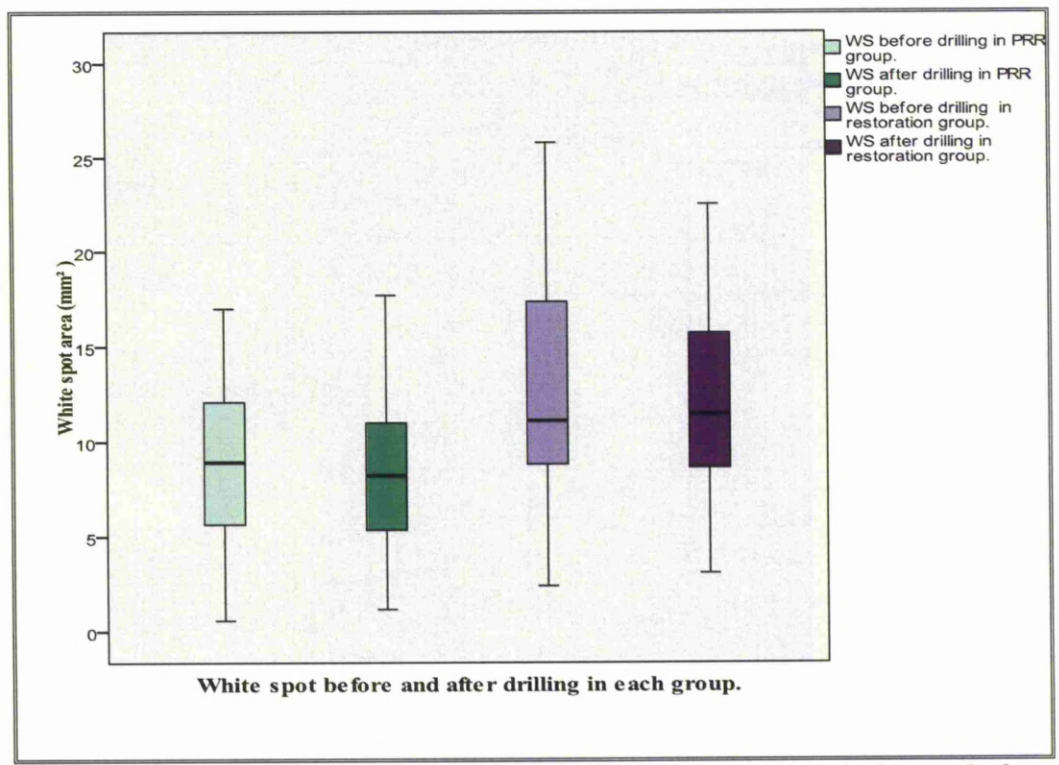


Figure 5.7: Box plot showing the white spot areas in each group before and after drilling teeth.

5.9 Hidden caries by QLF and Morita Cameras

The molar shown in Figure 5.8 best demonstrated how QLF and Morita systems can detect concealed occlusal decay. This tooth contained a "hidden" lesion, i.e., a caries lesion that has penetrated throughout the enamel into the dentine requiring restoration that cannot be seen by the dentist's eye and/or by the radiograph. Visual inspection in Figure 5.8-A was showing a tooth with a suspicious fissure. The radiograph (B) shows no apparent lesion. However, the QLF image (C) shows a loss of fluorescence suggesting a subsurface caries. The Morita image shows red fluorescence (D). Since QLF and Morita images show the mineral loss and the bacterial activity in the same areas on the occlusal surface of the tooth exactly they indicated that there was something that needed to be investigated. The presence of this lesion was confirmed by drilling the tooth (Figure 5.8-E, F and G) and these images showed the amount of tissue destruction occurring in this tooth and the decision made by clinicians whether monitoring and/or simple application of fluoride as a preventive measure was required.

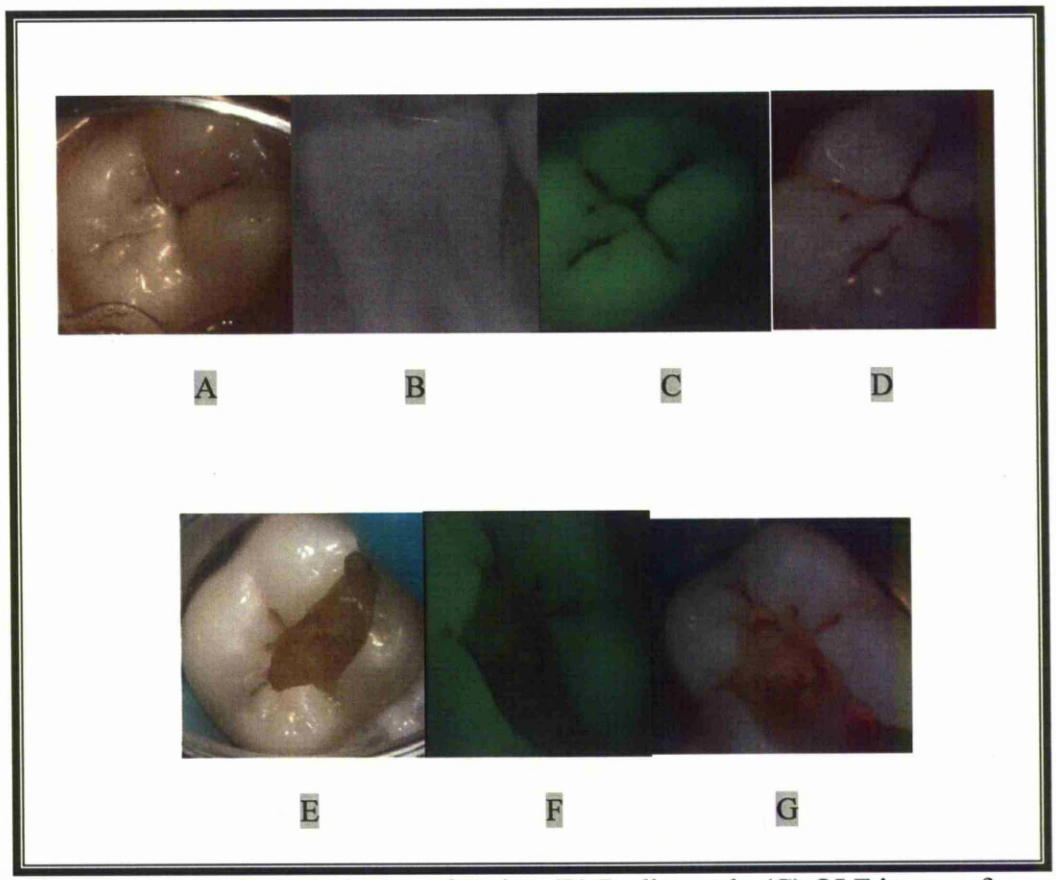


Figure 5.8: (A) White light image of molar. (B) Radiograph, (C) QLF image of tooth showing localised areas of decay (dark spot), (D) Morita image showing red fluorescence, (E) White light image after the tooth has been drilled and some of the caries removed, (F) QLF image showing the amount of demineralised tissue after drilling, (G) Morita image after drilling.

5.10 Residual caries by QLF and Morita Cameras

One of the most important observations in this study was that if the clinician decided to investigate and open the tooth then not all the demineralised tissue was removed; this means that there was residual demineralised tissue or caries underneath and/or around the sealant or restoration. Figure 5.9 shows the amount of demineralised tissue remaining after a completion of a cavity and after the placement of a restoration.

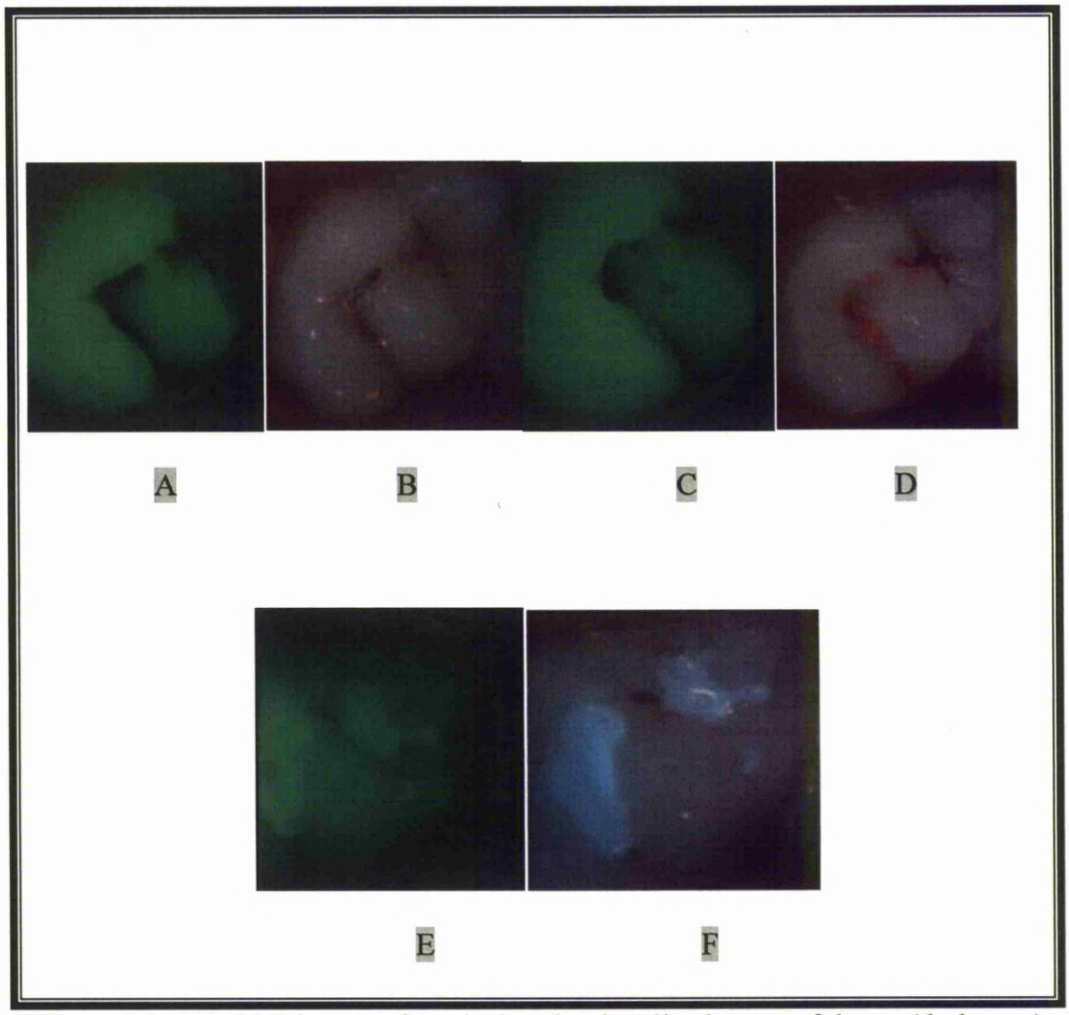


Figure 5.9: (A) QLF image of tooth showing localised areas of decay (dark spot). (B) Morita image showing red fluorescence, (C) QLF image showing the amount of demineralised tissue remaining, (D) Morita image after cavity preparation, (E) QLF image for the final restoration and there was still area of mineral loss (dark spots) around the restoration, (F) Morita image for final restoration.

Another further example of a cavity preparation where demineralised tissue has not been fully removed can be seen in Figure 5.10

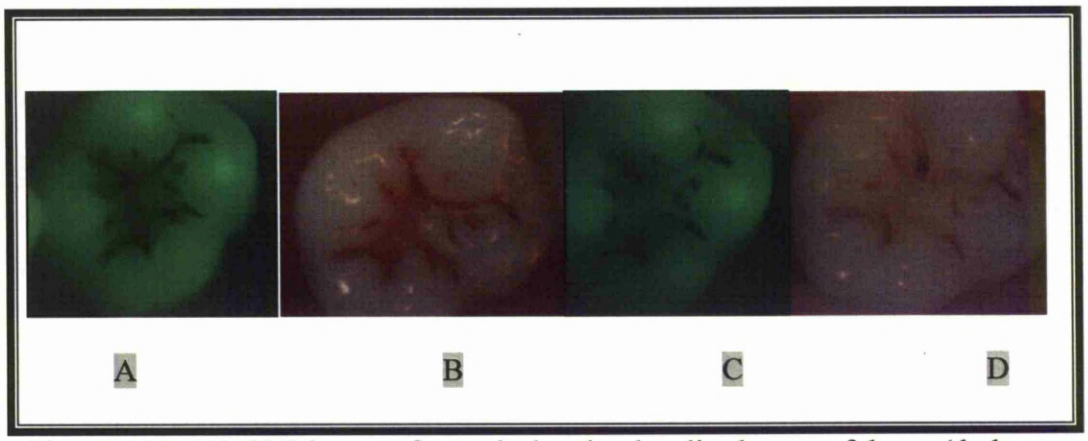


Figure 5.10: (A) QLF image of a tooth showing localised areas of decay (dark spot), (B) Morita image showing red fluorescence, (C) QLF image showing the amount of demineralised tissue remaining, (D) Morita image for the completed cavity preparation.

5.11 Preliminary Index for Occlusal Caries Diagnosis

Following completion of this study it was possible to use the data to develop a guide to give the clinician additional objective information to aid decision making. The mean values and confidence intervals for each QLF parameter were calculated and index was devised (Table 5.7).

Table 5.7: A preliminary index for clinical management of early occlusal caries.

Treatment Decision	ΔF	ΔQ	ΔR Morita	ΔR QLF	WS area
PRR or Fissure Sealant	12-23	100-260	30-49	23-30	1-12
Simple class I occlusal Restoration	24-32	261-400	50-64	31-40	13-20

5.12 Discussion

Taking into consideration the often slow progression of dental caries and the desire of the oral healthcare professional to prevent or minimise caries progression, it has become more important to record information for not only cavitated lesions, but also for the non-cavitated early stages of dental decay (Pitts and Stamm, 2004).

Combinations of visual examination and probing were the mainstay of occlusal caries diagnosis for years. Probing pressure was shown to potentially damage the fissures (Bergman and Lindén, 1969; Ekstrand et al., 1987; Van Dorp et al., 1988). Unfortunately, visual inspection alone as a diagnostic means may leave much undetected dental caries in its early phases. The QLF technique has been reported to be a valuable device for early detection, quantification, and monitoring of non-cavitated caries lesions (De Josselin de Jong et al., 1995; Kuhnisch and Heinrich-Weltzien, 2004; Pretty et al., 2003d). The *in vitro* study and the first *in vivo* study in this thesis showed promise for the new visual QLF system and has been validated on the occlusal surfaces in *vitro* and *in vivo* with histological examination in the laboratory.

The nature of the present study clearly precluded histological validation of diagnostic accuracy. This was the first study to examine the ability of QLF to detect early occlusal caries in the clinical situation, which is an accepted challenge for the dental clinician. Generally, during basic clinical examination and charting this hardly visible discoloration or white spot on occlusal surface are clinically

negligible (Kuhnisch et al., 2007). The results of this study have shown that the QLF system would not only be a suitable tool to use in dental clinic to detect the mineral loss in early carious lesions, but it would also be applicable in supporting the diagnostic opinion and the type of management required on occlusal tooth surface. The validity, reproducibility and sensitivity of the QLF technique for detection and quantification of caries in enamel have been assessed with previously used caries quantification techniques such as chemical analysis, transverse microradiography and laser-induced fluorescence as well as longitudinal microradiography (Al-Khateeb et al., 1997; Ferreira Zandona et al., 1998b; Hafstrom-Bjorkman et al., 1992; Lagerweij et al., 1999), and optical coherence tomography (Amaechi et al., 2003).

A standard analysis technique was employed throughout the study to reduce the subjectivity. Images of teeth were reassessed after a two weeks interval and, the intra-examiner value was 0.91. Sound tissue fluorescence levels were compared with the actual fluorescence values obtained at a threshold level of 5%. This allows any change between sound and demineralised enamel under a 5% fluorescent change to be ignored, and as a result removes any "noise" from the system and facilitates reliable and consistent analysis (Pretty et al., 2002b). The results showed that the clinician can rely on ΔF, ΔQ values for QLF device and ΔR Morita values in order to reach a definite decision about the diagnosis and the degree of early restorative intervention required as these values showed a statistically significant difference at the baseline between the two treatment groups ($p<0.05$). The reduction in the mean ΔF and ΔQ values in the FS/PRR group after the drilling was

expected in this type of restoration as the most demineralised fissures and grooves were removed. On the other hand, in the class I restorations group the mean of ΔF and ΔQ values increased after drilling as the carious and the demineralised tissue increased with increasing depth.

After completion of the analysis of ΔR both for QLF and Morita images it was found that there were outliers (Figure 5.5 and 5.6). In statistics, an outlier is "an observation that is numerically distant from the rest of the data in them" and outliers can occur by chance in any distribution (Barnett and Lewis, 1994). In this case it may be explained by the fact that patients' teeth were imaged without cleaning them. The red fluorescence observed will therefore include not only any intrinsic fluorescence of the tooth but also any plaque and food debris compacted into the occlusal pits and fissures. The majority of red fluorescence can be removed from the tooth by careful professional cleaning. Nevertheless, red fluorescence will persist even following cleaning if there has been bacterial invasion and bacterial activity within the tooth tissues (Waller et al., 2003). In addition, heavy dental plaque deposits on teeth fluoresce red or bright orange (Van der Veen and De Josselin de Jong, 2000).

It has been proposed that the fluorescence may be due to the presence of porphyrins in certain oral plaque species (Pretty et al., 2005), mainly Gram-negative anaerobes, which are more numerous in mature plaque (Marsh and Martin, 2009). Results showed that the mean ΔR increased after drilling in the FS/PRR group as well as in the simple class I occlusal restoration group for both

the QLF and Morita systems. This may be explained as following, as the tooth was drilled a new surface was exposed and the red fluorescence increased because both systems detected the red fluorescence of the superficial surface and the red fluorescence in the newly exposed surface which was also infected by the bacteria. It could be clearly seen that the Morita camera detected more red fluorescence than the QLF camera. This can be explained by the fact that filters in Morita camera were designed to detect only red rather than those in the QLF system which were primerly designed for mineral loss and gain. An unexpected additional benefit of QLF was that bacterial red fluorescence could also be visualised.

As stated previously, the teeth had radiographs with a radiographic index of zero confirmed by the supervisors (radiographs do not show any caries) and verified again by a researcher in this study following careful examination of the dental radiograph. This confirmed that the radiographs did not show any early enamel lesions. Radiographs are known to have a higher specificity than sensitivity, which means that false negatives diagnosis is more likely to occur in the presence of the disease. This may result in further development of the disease leading to irreversible changes to healthy teeth (Dove, 2001). Radiographs are useful to support the detection of occlusal caries but only if the disease has already progressed into dentine. A carious lesion visible on a radiograph is known to be significantly more infected with *lactobacilli* and *Streptococcus mutans* than a non-X-ray-detectable lesion (Lussi et al., 2001). Therefore, radiography is not appropriate for early detection of dental caries on the occlusal surface of the teeth.

Accordingly, occlusal sites should be examined frequently for signs of caries with tools other than the conventional methods. QLF can detect about twice as many demineralised precavitated enamel areas than a conventional visual examination or any other caries detection instruments (Stookey, 2005). Studies conducted in Germany found that the QLF system identified more non-cavitated caries lesions as well as considerably smaller lesions than visual inspection (Kuhnisch et al., 2007).

Occlusal caries diagnosis is difficult to achieve since there may be dentine caries beneath apparently sound surfaces. In this study "hidden" caries was found in 10% of molars after exposing the tooth. The study demonstrated the potential benefit of QLF for detecting occlusal decay and in teeth which contained a "hidden" lesion. The data from this study has shown that by imaging the teeth with the QLF and Morita systems, that information regarding subsurface lesions can be used to indicate mineral loss (dental decay) that is not detectable by radiographs. In addition with the preliminary index developed, it will provide valuable information so that the clinician can decide whether restorative intervention is required or whether less invasive action is more appropriate, perhaps with longitudinal monitoring.

One of the most important observations in this study was that the ability of QLF and Morita images in showing residual demineralised tissue or caries underneath and/or around the sealant or restoration which is very important in terms of the patients' oral health. In the example presented in Figure 5.10; it can be seen clearly that the clinician concentrated more on the cavity body of the lesion itself more

than its margins in the adjacent fissures and groove, reflecting the difficulty of recognising demineralised tissue in marginal areas when relying only on visual and traditional clinical techniques.

The dilemma of imperfect excavation in conventional preparation techniques (residual or secondary caries) has been found to be one of the most frequent causes for re-restoration (Mjor, 1985). Although residual caries does not seem to be the sole criterion for re-restoration, it is clear that every effort is made to eradicate carious tissue as much as possible (Weerheijm and Groen, 1999). Complete removal of carious dental tissues before restoring the tooth is recommended (Weerheijm et al., 1999) but this in practice is difficult to achive. The solidity and the colour of the dental tissues after complete caries excavations are currently the key factors for the dental professional to distinguish between carious and non carious tissues (Banerjee et al., 2000; Kidd et al., 1993a). This is however, highly subjective and leads to carious areas being left and the judgment as to whether caries removal is complete is often not easy (Lennon et al., 2002). This aspect of the study showed the importance of the use of the QLF and Morita system to check whether during cavity preparation and before placement of any restorative material, that all carious tissue has been removed. The data presented in this study demonstrated the importance of fissure sealants. It has been confirmed recently that sealing has to be considered as an integral part of the management and treatment scale for caries ranging from non-operative approach to restoration, especially in permenant molars (Splieth et al., 2010).

Decisions on treatment's requirements for any dental lesion are based on a number of criteria. These include patient's oral hygiene, type and frequency of diet consumed, capability to attend appointments and changes to habits and behaviour to improve dental health. All of these aspects dentists recognise, after careful assessment and history taking (Walmsley et al., 2007).

Although major advantages of the optical systems employed in the current study were found, certain difficulties were encountered included difficulty in accessing occlusal surfaces. This was due to the relatively large head of the QLF handpiece, depth of field of the QLF camera. Additionally, in clinical situations where rubber dams are used, the tooth to be examined is clamped and in this situation the QLF head cannot be positioned horizontally above the tooth. This means that the QLF head is too far away from the tooth to be imaged and the light intensity is decreased resulting in unacceptable imge quality. If the head of the device was smaller, would lead to improved orientation and accessibility of the required tooth surfaces and allow better quality image to be made. If the QLF is to be useful for all surfaces of the tooth, investement in improving the design would be beneficial. It would be worth considering the use of fibre optic technologies.

5.13 Conclusion

Within the limitation of this study, it is possible to conclude that the QLF system was able to detect demineralisation on occlusal surfaces earlier than current

conventional diagnostic methods used in everyday dental practice. Higher values of ΔF and ΔQ were associated with more extensive restoration. Higher values of ΔR Morita were associated with more advanced and active dental lesions. ΔF, ΔQ and ΔR values may be useful in aiding clinical diagnosis and supporting decision making in relation to the restorative management of occlusal caries. QLF technology could aid the dental clinicians not only in early caries detection but also to help make diagnosis and to treat cases in more conservative way.

Therefore, QLF technology can be used to protect the teeth from undergoing a continuous cycle of restorative treatment which often resulted when dental caries is discovered at advanced stages (for example when dentinal caries is very close to the pulp). QLF also provides visual and quantitative feedback to patients. It is a rapid, non-invasive quantifiable method of caries detection with good potential for incorporation into clinical diagnosis and management regimes. It is evastigated that, in the future it will provide dentists with information necessary to provide preventive advice with minimal early intervention and less tissue destruction. It can be concluded that QLF is an appropriate method to provide additional information, not currently available using conventional technique which may be used to make the diagnosis of caries on occlusal tooth surfaces less subjective. QLF can be used as an aid in concealed and residual caries detection before final placement of restorations. Finally, this tool has considerable application for use in longitudinal monitoring for assessing the outcome of preventive treatments.

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CHAPTER 6

GENERAL CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

6.1 Conclusions

The reproducibility of QLF measurements has been high in both *in vitro* and *in vivo* clinical studies. Therefore, QLF has the potential for wider use in everyday clinical dentistry and the anticipated future use of QLF will markedly enhance the utility of this technology in clinical dental research and dental practice. Accordingly, my findings suggest that the QLF device could be used to support clinical management of dental patients when there is uncertainty about the clinical caries status of a tooth.

This device with its facilities for capturing images, storing them and longitudinal monitoring could facilitate collaboration between different centres, to enable experts' to give their opinion using this technology and the opportunity to send images and/or analysis by the internet.

It was found from the *in vitro* results that the QLF can be used to examine the histological sections with greater ease, and more distinct differentiation between the tissue involvements as it enhances the visual appearance.

Objective and evidence-based clinical decision making for caries management necessitates a diagnostic aid which can afford sensitive, reliable, specific and objective quantitative information regarding the lesions. All the indications from the results of this study suggest that QLF is capable of offering considerable improvements over currently used clinical methods and help particularly for the

early management of caries on the most challenging surfaces of the teeth, the occlusal (chewing) surfaces and guiding the operative management by ensuring that the cavity margins are not carious.

It has been demonstrated that the index developed *in vitro* differs to some extent from the one developed *in vivo*. It has been discussed that the storage time and conditions in the *in vitro* study affects the fluorescence results. For that reason, the index developed *in vivo* is recommended to be the QLF index of choice (Table 4.7) since all the variables were the same for all the teeth examined in both studies. From what has been found during conducting the *in vitro* study and red fluorescence it is essential to plan the work in such a way that any tooth collected need to be analysed immediately before any factors play a role in fluorescence loss. If this is difficult another suggestion is to leave each group in the same circumstances for the same fixed period this in turn will standardise the outcomes.

6.2 Suggestions for further studies

It is believed that demineralised areas in the tooth that show red fluorescence have progressed into a condition where the enamel arrangement is porous so that bacterial by-products or the bacteria themselves invade the lesion. Further work needs to be done in order to understand what red fluorescence really means, obtain more information about its nature and whether it can be removed. In addition, it will be interesting to monitor it and see if there is a relationship of red fluorescence on visibly sound tooth surfaces and hidden caries.

It has been found that, there is a good correlation of histology with the average fluorescence loss of teeth's sections after imaging by QLF. This suggests that it is worth investigating the use of QLF to quantify mineral loss in histological sections.

Further work is needed to define caries activity in more detail, validate the criteria and reliability on smooth surfaces. However, this early evaluation of the QLF classification system has found that the system is useful and has a good correlation with the histological examination.

It has also been found that QLF is able to show residual demineralised tissue or caries at cavity margins. This will be helpful to longitudinally monitor restoration margins after caries removal.

Further studies are necessary to evaluate other aspects of caries detection, approximal caries detection *in vitro* and under clinical conditions.

6.3 Suggestions for improvements to the QLF device and its software

Following the work in this thesis, the recommendations for the future development and modifications in QLF device are:

- Incorporation of the maximum fluorescence loss in the QLF software and automatically including this value in the data output, thus after analysis of a

lesion the clinician may be able to detect the deepest point of a lesion and obtain some reference of the actual depth.

- Modification of the red fluorescence detection and software analysis by QLF.

- To attain most favourable outcomes, fast and easier application; a smaller head size need to be developed to allow better access and easier assessment of the lesions.

- The use of an air spray directed across the mirror or the use of anti-fogging agents may reduce clouding of the mirror because of the breath of the patients. This will improve the image quality and reduce the time required to achieve good quality image.

- From the analysts's point of view, it is essential to offer a trolley with adjustable height to make it easier and more comfortable to analyse data. A considerable amount of time is spent analysing data from studies with large numbers of samples (teeth).

- One important point, to smooth the progress of the examination between different clinics in the same hospital or between different cubicles in the same clinic; the device should be provided with a rechargeable battery-powered device.

- The QLF device has many uses and should be shown to undergraduate students during their training consequently they become familiar with it and aware of its advantages. This would facilitate its introduction in the University Dental Hospitals first and then in the general dental practice when the students graduate.

- Develop the device in such away to facilitate the detection of proximal and hidden caries.

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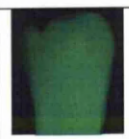
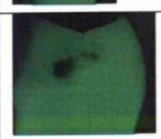
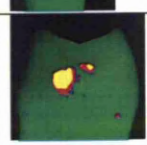
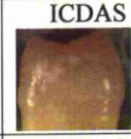
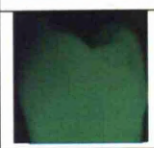
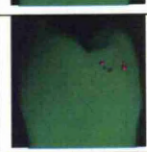
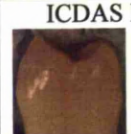
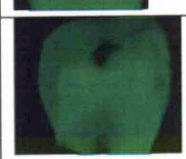
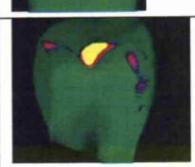
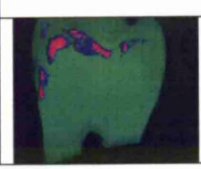
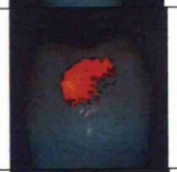
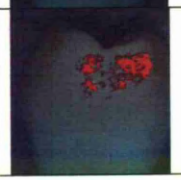
UNIVERSITY OF
LIVERPOOL

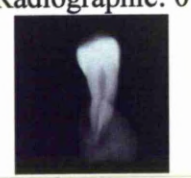
APPENDICES

APPENDIX 1
Data gathering from different indices and techniques employed for each tooth in *in vitro* study.

Tooth Number	QLF Images	QLF Lesion Analysis		Analysis Values		Index Score	
		WS	RF				
(T3)	Occlusal			ΔF[%] ΔQ[%.mm²] ΔR[%] Ws Area [mm²] RF Area[mm²]	23 179 63.9 7.79 3.34	ICDAS II 	**3**
	Buccal			ΔF[%] ΔQ[%.mm²] ΔR[%] Ws Area [mm²] RF Area[mm²]	8.43 1.7 22.2 0.2 0.47	ICDAS II 	**0**
	Lingual			ΔF[%] ΔQ[%.mm²] ΔR[%] Ws Area [mm²] RF Area[mm²]	18.7 6.74 22.3 0.36 1.6	ICDAS II 	**0**
	Mesial			ΔF[%] ΔQ[%.mm²] ΔR[%] Ws Area [mm²] RF Area[mm²]	31.2 148 30.6 4.73 4.84	ICDAS II 	**0**
	Distal			ΔF[%] ΔQ[%.mm²] ΔR[%] Ws Area [mm²] RF Area[mm²]	9.84 3.78 24 0.38 2.56	ICDAS II 	**0**
Histological sections examined with QLF	First half			ΔF[%] ΔQ[%.mm²] ΔR[%] Ws Area [mm²] RF Area[mm²]	24.6 77.1 0 3.14 0		Histological 2
	Second half			ΔF[%] ΔQ[%.mm²] ΔR[%] Ws Area [mm²] RF Area[mm²]	9.55 36.6 0 3.84 0		

Tooth Number (T3)	Morita Images	Morita Analysis RF	Analysis Values	
	Occlusal	 	ΔR[%] RF Area [mm²]	79.5 5.3
	Buccal	 	ΔR[%] RF Area [mm²]	36.4 2.18
	Lingual	 	ΔR[%] RF Area [mm²]	33.4 0.96
	Mesial	 	ΔR[%] RF Area [mm²]	49.3 3.77
	Distal	 	ΔR[%] RF Area [mm²]	31.1 2.67

Radiographic: 0



APPENDIX 2

University of Liverpool Sponsor Letter (*In vivo* study 1)

THE UNIVERSITY
of LIVERPOOL

I M Carter, BSc, PhD, CEng, MIEE, MCMI
Director of Research

Research and Business Services

The Foresight Centre
3 Brownlow Street
Liverpool L69 3GL

Telephone: 0151 794 8723
Facsimile: 0151 794 8728
Email: i.carter@liv.ac.uk

Ref: SP000214

UOL000198

25th January 2007

Professor S Higham
School of Dental Sciences

Dear Professor Higham

I am pleased to confirm that, the University will act as Sponsor under the DoH Research Governance Framework for Health and Social Care for your study entitled "Development of Caries Indices Using Quantitative Light-induced Fluorescence(QLF).". In accepting this role the University expects you, as Chief Investigator, to conduct the project in full compliance with the requirements of the Framework so that it is able to meet its obligations as Sponsor.

Having consulted the insurance broker, I also confirm that the University professional indemnity and clinical trials insurances will apply to the project as appropriate. The University does provide cover for non-negligent harm and this must be made clear to prospective participants in the work.

I trust that this statement will enable you to proceed with your project. Please let me know if I can be of any further assistance in this matter.

Yours sincerely,

Cc Head, School of Dental Sciences
Mrs L Carter, Research Administrator, Faculty of Medicine Office

University switchboard
Telephone: 0151 794 2000
Facsimile: 0151 708 6502

APPENDIX 3
Liverpool (Adult) Local Research Ethical Committee Approval
(*In vivo* study 1)

NHS
National Research Ethics Service

Liverpool (Adult) Local Research Ethics Committee
1 Arthouse Square
61-69 Seel Street
Liverpool
L1 4AZ

Telephone: 0151 296 7541
Facsimile: 0151 296 7536

11 April 2007

Professor Susan M Higham
Professor of Oral Biology
The University of Liverpool
Edwards Building
Daulby Street
Liverpool
L69 3GN

Dear Professor Higham

Full title of study: **Development of Caries Indices Using Quantitative Light-induced Fluorescence(QLF).**
REC reference number: **07/Q1505/23**

The Research Ethics Committee reviewed the above application at the meeting held on 04 April 2007. Thank you for attending to discuss the study.

Ethical opinion

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation.

The favourable opinion is subject to the following amendments/clarifications:

- The members suggest that the poster title should simply be 'Tooth Decay Study', drawing the patients' attention to the information sheets they were given upon registration asking them to consider participating in the study
- Please entitle the Patient Information Sheet 'Patient Information Sheet' with the subtitle 'A Study into the use of Quantitative Light Induced Fluorescence to measure Tooth Decay (caries)'
- Please amend the grammar in the Patient Information Sheet to more user-friendly, lay terms. It should refer to tooth decay and not just 'caries', and should refer to the study as a study and not a trial.
- Please amend the Consent Form to carry the same title as the Patient Information Sheet.

Ethical review of research sites

The Committee agreed that all sites in this study should be exempt from site-specific assessment (SSA). There is no need to submit the Site-Specific Information Form to any Research Ethics Committee. The favourable opinion for the study applies to all sites involved in the research.

Conditions of approval

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Application	5.3	08 March 2007
Investigator CV		14 October 2006
Protocol	1.2	16 November 2006
Covering Letter		09 March 2007
Letter from Sponsor		25 January 2007
Advertisement	1 3	16 December 2006
Participant Information Sheet	1.2	18 November 2006
Participant Consent Form	1 1	18 November 2006
C.V. For Supervisor		15 December 2006

R&D approval

You should arrange for the R&D office at all relevant NHS care organisations to be notified that the research will be taking place, and provide a copy of the REC application, the protocol and this letter.

All researchers and research collaborators who will be participating in the research at a NHS site must obtain final approval from the R&D office before commencing any research procedures.

Membership of the Committee

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

07/Q1505/23	Please quote this number on all correspondence

With the Committee's best wishes for the success of this project

Yours sincerely

Dr Taj Purewal
Chair

Email: Ronald.wall@liverpoolpct.nhs.uk

Enclosures: *List of names and professions of members who were present at the meeting and those who submitted written comments*

Copy to: *Dr Ian Carter*
Research and Business Services
The University of Liverpool

APPENDIX 4

NHS Research and Development Approval

(*In vivo* study 1)

Prof Sue M Higham
Professor of Oral Biology
University of Liverpool
Dental Hospital
2nd Floor Edwards Building
Daulby Street
Liverpool

Wednesday 25th April 2007

Dear Prof Higham

A Study into the use of Quantitative Light-induced Fluorescence (QLF) to measure Tooth Decay (Caries) - Trust Study No.3427

I can confirm that the pre-trial documentation for this study is now complete. The study has received approval from the appropriate Ethics Committee and the financial arrangements meet the NHS executive requirements.

I can confirm, therefore, that the Trust is happy for you to conduct this work on its premises provided that the study is conducted in compliance with the Research Governance Framework 2005.

Please contact Jaime Halvorsen in the R&D Directorate (Jamie.Halvorsen@rlbuht.nhs.uk, 4th floor Linda McCartney Centre, Ext 3771) when you commence your study.

Yours sincerely

Jacqueline A Pirmohamed
R&D Manager
Jacqui.Pirmohamed@rlbuht.nhs.uk

APPENDIX 5

Poster

(*In vivo* study 1)

Tooth Decay Study

When you registered today you will have received information about the Tooth Decay Study, please read it and tell us if you would like to take part in the study.
If you are interested please contact The Reception Desk before being called.
Thank you.

Version1.4
Date 17.04.2007

APPENDIX 6

Patient Information Sheet

(*In vivo* study 1)

UNIVERSITY OF LIVERPOOL

School of Dental Sciences
Liverpool University Dental Hospital and School of Dentistry
Pembroke Place, Liverpool L3 5PS

Version 1.3
Date 17.04.2007

Patient Information Sheet

A Study into the use of Quantitative Light-induced Fluorescence (QLF) to measure Tooth Decay (Caries)

What is the purpose of the study?
We are currently developing a new method of diagnosing and classifying tooth decay (caries) on teeth. We will study and examine the extent of the disease, its location and severity.

Has the study been approved?
Yes. A local research ethics committee have approved this study.

Who is paying for the study?
The Cariology Research Group in the School of Dental Science, University of Liverpool.

Who will be conducting the study?
The study is being run by Professor Susan Higham, Professor of Oral Biology. Dr Colette Balmer, Clinical Lecturer, Acting Consultant in Oral Surgery and Associate Postgraduate Dental Dean Dr. Philip Smith, Senior Lecturer and Consultant in Restorative Dentistry, and Mrs.Manal Alammari, PhD student.

Why have I been asked to take part?
You have been asked because we want healthy adult volunteers.

What will I have to do?
If you have been invited to take part in the study, you will be examined; images of your teeth will be taken with a digital camera and also with a special dental camera which uses a blue light - (QLF), which allows dentists to detect teeth that have very early decay that can not be seen by eye. If

any of your teeth need to be extracted (removed), we will keep them for further investigation and examination.

How long will the study last?
The examination involving digital and QLF will last approximately 20 minutes.

Will I be recompensed for my time?
No

What if I don't want to take part?
It is important that you understand that joining this study is entirely voluntary. You should not feel obliged to take part, and you do not have to give a reason if you don't want to. If you do take part in the study, but, later decide that you don't want to continue you can also withdraw at any time without giving a reason. Your decision will not affect your treatment in any way.

What if I have a question or there is a problem on the trial?
If you have any questions or problems you can speak with one of the dentists running the study in the hospital. They can be reached by telephone on 07791233481 and arrangements can be made to deal with the problem, or you can email them (manal @liverpool.ac.uk, p.w.smith@liverpool.ac.uk, s.m.higham@liverpool.ac.uk, Colette.Balmer@rlbuht.nhs.uk).

Are there any potential side effects?
This study only involves examination and imaging which has no side effects.

What do I do if I want to take part?
If you would like to take part, please sign all the relevant sections of the consent form that you will have been given. All information about you will be kept secure and confidential. As soon as we have collected the necessary data, all information which identifies you will be removed and replaced by a code.

APPENDIX 7

Consent Form

(*In vivo* study 1)

UNIVERSITY OF LIVERPOOL

Version 1.2
Date 17.04.2007

CONSENT FORM

A Study into the use of Quantitative Light-induced Fluorescence (QLF) to measure Tooth Decay (Caries).

Volunteer's name _____

To be completed by the volunteer

Have you read the information sheet	Yes / No
Have you had an opportunity to ask any questions	Yes / No
Are you satisfied with the answers	Yes / No
Do you feel you have enough information about the study	Yes / No
Do you understand that you can withdraw from the study at any time without having to give a reason	Yes / No
Have you been supplied with the emergency phone number	Yes / No
I am happy to participate in the study	Yes / No

Signed by the volunteer _____ Date _____

Signed by the investigator _____Name of the investigator_____

APPENDIX 8

Patient Clinical Chart

(*In vivo* study 1)

Caries Indices Clinical Study

Date: _____

Patient Study Code: _____

Tooth Number: ___ ___ ___

Consent form signed ☐

Clinical Examination ICDAS Scoring:

	Tooth #	**Tooth #**	**Tooth #**
Occlusal	_____	_____	_____
Buccal	_____	_____	_____
Lingual	_____	_____	_____
Rad.Index	_____	_____	_____

Prophylaxis:

☐ No

☐ Yes

☐ White Digital Images of 3 surfaces Obtained.
☐ QLF images for 3 Surfaces Obtained.
☐ Morita Camera images obtained.
☐ Image for the radiograph.

Tooth Extracted

☐ No

☐ Yes

Comments: _____

APPENDIX 9

Statistical Consultation Letter

(In vivo study 2)

UNIVERSITY OF LIVERPOOL

School of Dental Sciences
University of Liverpool
Pembroke Place
Liverpool
L3 5PS

T 0151 706 5113
F 0151 706 5250
E g.burnside@liv.ac.uk

www.liv.ac.uk

To whom it may concern,

Re: Quantitative light-induced fluorescence (QLF): A potential tool for early occlusal dental caries detection and supporting decision making *in vivo*.

I have performed the sample size calculation for this study, which is detailed below.

Sample size calculation
The sample size has been calculated to allow detection of a statistically significant difference in **Δ F** between teeth which are found to have carious lesions and require restoration, and those which are not having carious lesions and needs only Preventive Resin Restorations, using a t-test.
The minimum clinically significant difference between groups in **Δ F** has been set at a value of 5. The standard deviation has been estimated at 5.9; using data from an in vitro study. The power of the study is set at 80%, and α is set at 0.05. Using these values, the calculation produces a minimum sample size of 23 subjects per group.

Yours faithfully,

Girvan Burnside
Lecturer in Dental Statistics

APPENDIX 10

University of Liverpool Sponsor Letter

(*In vivo study* 2)

UNIVERSITY OF LIVERPOOL

J F Fox, BA, MPA, PG Dip in Law
Manager, Contract Services
Research and Business Services

The Foresight Building
3 Brownlow Street
Liverpool L69 3GL

Telephone: 0151 794 8793
Facsimile: 0151 794 8728
Email: j.f.fox@liverpool.ac.uk

RBS Ref: SP000319
Faculty Ref: UOL000340

Wednesday, 07 May 2008

Dr Philip Smith (Student Investigator: Manal R Alammari)
School of Dental Sciences

Dear Dr Smith

I am pleased to confirm that the University will act as Co-Sponsor with the Royal Liverpool and Broadgreen Hospitals NHS Trust under the Department of Health's Research Governance Framework for Health and Social Care for your study entitled "Quantitative light-induced fluorescence (QLF): A potential tool for early occlusal dental caries detection and supporting decision making in vivo". In accepting this role the University expects you, as Chief Investigator, to conduct the project in full compliance with the requirements of the Framework so that it is able to meet its obligations as Co-Sponsor.

Having consulted the insurance broker, I also confirm that the University professional indemnity and clinical trials insurances will apply to the project as appropriate. The University does provide cover for non-negligent harm and this should be made clear to any prospective participants in the work.

I trust that this statement will enable you to proceed with your project. Please let me know if I can be of any further assistance in this matter.

Yours sincerely,

Mr James Fox
Manager, Contract Services

Cc Mrs Lindsay Carter, Research Coordinator, Faculty of Medicine Support Office

APPENDIX 11

Liverpool (Adult) Local Research Ethical Committee Approval

(*In vivo study* 2)

Liverpool (Adult) Research Ethics Committee
Bishop Goss Complex
Victoria Building
Rose Place
Liverpool
L3 3AN

Telephone: 0151 330 2077
Facsimile: 0151 330 2075

09 June 2008

Dr Philip Smith
Senior Lecturer and Consultant in Restorative Dentistry
University of Liverpool School of Dental Science
Room 4.23, Dental Hospital, Pembroke Place
Liverpool
L3 5PS

Dear Dr Smith

Full title of study: **Quantitative light-induced fluorescence (QLF): A potential tool for early occlusal dental caries detection and supporting decision making in vivo.**

REC reference number: **08/H1005/50**

The Research Ethics Committee reviewed the above application at the meeting held on 04 June 2008. Thank you for attending to discuss the study.

Ethical opinion

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Ethical review of research sites

The favourable opinion applies to the research site listed on the attached form.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission at NHS sites ("R&D approval") should be obtained from the relevant care organisation(s) in accordance with NHS research governance arrangements. Guidance on applying for NHS permission is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
Application	5.6	08 May 2008
Investigator CV		08 May 2008
Protocol	1.1	14 March 2008
Covering Letter		08 May 2008
Letter from Sponsor		07 May 2008
Statistician Comments		07 May 2008
Participant Information Sheet	1.1	14 March 2008
Participant Consent Form	1.1	14 March 2008
C.V. for Supervisor		14 October 2007

Membership of the Committee

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Now that you have completed the application process please visit the National Research Ethics Website > After Review

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email referencegroup@nres.npsa.nhs.uk.

08/H1005/50	Please quote this number on all correspondence

08/H1005/50

With the Committee's best wishes for the success of this project.

Yours sincerely

Dr Tej Purewal
Chair

Email: Ronald.Wall@liverpoolpct.nhs.uk

Enclosures: *List of names and professions of members who were present at the meeting and those who submitted written comments*
"After ethical review – guidance for researchers
Site approval form (SF1)

Copy to: *Dr Ian M Carter, University of Liverpool*

Liverpool (Adult) Research Ethics Committee
LIST OF SITES WITH A FAVOURABLE ETHICAL OPINION

For all studies requiring site-specific assessment, this form is issued by the main REC to the Chief Investigator and sponsor with the favourable opinion letter and following subsequent notifications from site assessors. For issue 2 onwards, all sites with a favourable opinion are listed, adding the new sites approved.

REC reference number:	08/H1005/50	**Issue number:**	0	**Date of issue:**	09 June 2008
Chief Investigator:	Dr Philip Smith				
Full title of study:	Quantitative light-induced fluorescence (QLF): A potential tool for early occlusal dental caries detection and supporting decision making in vivo.				

This study was given a favourable ethical opinion by Liverpool (Adult) Research Ethics Committee on 04 June 2008. The favourable opinion is extended to each of the sites listed below. The research may commence at each NHS site when management approval from the relevant NHS care organisation has been confirmed.

Principal Investigator	*Post*	*Research site*	*Site assessor*	*Date of favourable opinion for this site*	*Notes* (1)
Dr Philip W Smith	Senior Lecturer/Consultant Restorative Dentistry.	Liverpool Dental Hospital, The University of Liverpool.	Liverpool (Adult) Research Ethics Committee	09/06/2008	

Approved by the Chair on behalf of the REC:

..... (Signature of Co-ordinator)

Ron White ..... (Name)

(1) *The notes column may be used by the main REC to record the early closure or withdrawal of a site (where notified by the Chief Investigator or sponsor), the suspension of termination of the favourable opinion for an individual site, or any other relevant development. The date should be recorded.*

APPENDIX 12

NHS Research and Development Approval

(*In vivo study* 2)

The Royal Liverpool and Broadgreen University Hospitals
NHS Trust

Royal Liverpool University Hospital
Prescot Street
Liverpool
L7 8XP

Tel: 0151 706 2000
Fax: 0151 706 5806

Manal Alammari
School of Dental Sciences
The Edwards Building
2nd Floor, Room 212
University of Liverpool
Daulby Street
L69 3GN

Wednesday 01 October 2008

Dear Alammari

Quantitative light-induced fluorescence (QLF): A potential tool for early occlusal dental caries detection and supporting decision making in vivo
Trust Study No.3658

I can confirm that the pre-trial documentation for this study is now complete. The study has received approval from the appropriate Ethics Committee and the financial arrangements meet the NHS executive requirements.

I can confirm, therefore, that the Trust is happy for you to conduct this work on its premises provided that the study is conducted in compliance with the Research Governance Framework 2005.

Please contact Jaime Halvorsen in the R+D Directorate (Jaime.Halvorsen@rlbuht.nhs.uk, 4th floor Linda McCartney Centre, Ext 3771) when you commence your study.

Yours sincerely

Dr Rosalind Kelly
R+D Manager (Acting)
Rosalind.Kelly@RLBUHT.nhs.uk

APPENDIX 13

Patient Information Sheet

(*In vivo study* 2)

UNIVERSITY OF LIVERPOOL

School of Dental Sciences
Liverpool University Dental Hospital
and School of Dentistry
Version 1.1
Pembroke Place, Liverpool L3 5PS

Date 14.03.2008

Patient Information Sheet

A Study into the use of Quantitative Light-induced Fluorescence (QLF) to Detect Tooth Decay (Caries)

What is the purpose of the study?
We are currently using a new method of diagnosing and classifying tooth decay (caries) on teeth. We will study and examine the presence, extent of the disease, its location and severity.

Has the study been approved?
Yes. A local research ethics committee have approved this study.

Who is paying for the study?
The Cariology Research Group in the School of Dental Science, University of Liverpool.

Who will be conducting the study?
The study is being run by Dr. Philip Smith, Senior Lecturer and Consultant in Restorative Dentistry. Professor Susan Higham, Professor of Oral Biology., and Mrs.Manal Alammari, PhD student.

Why have I been asked to take part?
You have been asked because we want healthy adult volunteers who are having decayed back teeth.

What will I have to do?
If you have been invited to take part in the study, your teeth will be examined; images of your teeth will be taken with a digital camera and also with a special dental camera which uses a blue light - (QLF), which allows dentists to detect teeth that have very early decay that can not be seen by eye.

How long will the study last?
The examination involving digital and QLF will last approximately 5-10 minutes.

Will I be recompensed for my time?
No

What if I don't want to take part?
It is important that you understand that joining this study is entirely voluntary. You should not feel obliged to take part, and you do not have to give a reason if you don't want to. If you do take part in the study, but, later decide that you don't want to continue you can also withdraw at any time without giving a reason. Your decision will not affect your treatment in any way.

What if I have a question or there is a problem on the trial?
If you have any questions or problems you can speak with one of the dentists running the study in the hospital. They can be reached by telephone on 07791233481 and arrangements can be made to deal with the problem, or you can email them (p.w.smith@liverpool.ac.uk, manal @liverpool.ac.uk , s.m.higham@liverpool.ac.uk).

Are there any potential side effects?
This study only involves examination and imaging which has no side effects.

What do I do if I want to take part?
If you would like to take part, please sign all the relevant sections of the consent form that you will have been given. All information about you will be kept secure and confidential. As soon as we have collected the necessary data, all information which identifies you will be removed and replaced by a code.

APPENDIX 14

Consent Form

(*In vivo study* 2)

UNIVERSITY OF LIVERPOOL

Version 1.1
Date 14.03.2008

CONSENT FORM

A Study into the use of Quantitative Light-induced Fluorescence (QLF) to Detect Tooth Decay (Caries).

Volunteer's name _____

Have you read the information sheet	Yes / No
Have you had an opportunity to ask any questions	Yes / No
Are you satisfied with the answers	Yes / No
Do you feel you have enough information about the study	Yes / No
Do you understand that you can withdraw from the study at any time without having to give a reason	Yes / No
Have you been supplied with the contact phone number	Yes / No
I am happy to participate in the study	Yes / No

Signed by the volunteer _____ Date _____

Name of the investigator _____ __ Signature_____

APPENDIX 15

Patient Clinical Chart

(*In vivo study* 2)

Caries Indices Clinical Study 2

Date: _____

Patient Study Code: _____

Tooth Number: ____ ____ ____ ____

Consent form signed ☐

Clinical Examination ICDAS Scoring:
Occlusal_____

☐ White Digital Images of occlusal surface Obtained before & after drilling.
☐ Image for the radiograph. RI: _____.
☐ QLF images for occlusal surface Obtained before & after drilling.
☐ Morita Camera images for occlusal surface Obtained before & after drilling.

Tooth Drilled?

☐ No

☐ Yes Filled by_____.

Comments:

_____

_____

APPENDIX 16

Development of Caries Indices Using Quantitative Light-induced Fluorescence (QLF) *in vitro*.

M.R Alammari, P.W Smith, E.de Josselin de Jong, S.M Higham.

Introduction:

The prevalence of dental caries overall has declined in many European countries but this has not occurred equally for all tooth surfaces. This makes site specific diagnosis of dental caries more important, especially on the occlusal surfaces which are commonly affected. Several methods of caries diagnosis have been used but most rely on the dentist's subjective interpretation of clinical findings.

The objective of investigation:

Currently, an objective and a well-defined process for classifying carious lesions clinically by QLF does not exist. The aims of this study were to: use QLF to quantify the extent of mineral loss in caries lesions using the histological gold standard and to develop an interpretative clinical index of QLF values.

Method:

100 unrestored extracted human posterior teeth were cleaned, clinical research methods ICDAS II scoring were used, images of five surfaces (occlusal, buccal, lingual, mesial and distal) for each tooth using different camera systems (White-light digital camera, QLF and Morita camera), periapical radiographs, histology, TMR (Transverse Micro-radiography) and Micro CT (Microcomputed tomography) were employed. All the information gathered and analysed with special software.

Results:

QLF scores were significantly different at ICDAS scores 1, 2, 3 and 4($p < 0.001$). A QLF Index was developed to classify early carious lesions by the use of range of delta F at 5% and 95% Confidence Interval for Mean with the histology.

Conclusion:

Early indications suggest that QLF may enhance the identification of early demineralisation on the occlusal surface and plaque red fluorescence in carious lesions while images from Morita camera gives information regarding red fluorescence only. QLF was able to identify caries on the occlusal surface and differentiate between lesions of varying severity in early stages. It is anticipated that QLF will be valuable tool in routine clinical practice and reduce the patient's exposure to ionising radiation.

APPENDIX 17

Early occlusal caries management supported by Quantitative Light-induced Fluorescence (QLF).

M.R. Alammari, P.W. Smith, E.de Josselin de Jong, S.M. Higham.

School of Dental Sciences, University of Liverpool, Liverpool, UK.

An increased proportion of the total caries burden is found in fissures and subsequent restorative repair is costly in terms of time, resources and oral health. Caries diagnosis is important not only in preventive caries strategies but also in minimising and guiding operative interventions. The objective of this study was to determine whether the QLF parameters ΔF and ΔQ were appropriate for aiding diagnosis and clinical decision making of early occlusal caries by comparing QLF analysis with actual restorative management. Following ethical approval 46 subjects attending a dental teaching hospital were enrolled into the study. White light digital (WL) and QLF images/analyses of 46 unrestored posterior teeth with suspected occlusal caries were made after a clinical decision had already been taken to explore the fissure operatively. WL and QLF imaging/analysis were repeated after initial cavity preparation, and the type of restorative treatment was determined by the supervising clinician independent of any imaging performed. The actual restorative management carried out was recorded as a fissure sealant/ preventive resin restoration (FS) or a class I occlusal restoration (Rest.) thus reflecting the extent of operative intervention. All QLF images obtained were analysed independently without knowing the treatment outcome. The results showed statistically significant differences between the two treatment groups ΔF ($p= 0.002$) (mean ± SD: 22.60 ± 5.60-FS and 28.80 ± 6.06-Rest.) and ΔQ ($p= 0.012$) (mean ± SD: 230.49 ±161.82-FS and 348.30 ± 235.94-Rest.) thus higher values of ΔF and ΔQ were associated with more extensive restoration. ΔF and ΔQ values may be useful in aiding clinical diagnosis and supporting decision making in relation to the restorative management of occlusal caries.

APPENDIX 18

Development of A Clinical Caries Index Using Quantitative Light-induced Fluorescence.

M.R. Alammari, S.M. Higham, E.de Josselin de Jong, M.C. Balmer, P.W Smith.

School of Dental Sciences, University of Liverpool, Liverpool, UK.

Introduction: Dental caries still remains one of the most prevalent diseases. Currently clinical assessment of caries is based on subjective qualitative methods, and there is a need for an objective and well-defined process for classifying caries lesions. Techniques that allow visualisation of mineral changes in the caries process, such as Quantitative Light-induced Fluorescence (QLF), appear to have potential in this regard.

Objectives: The aim was to develop and validate a clinically applicable index that relates green fluorescence loss (ΔF and ΔQ) together with the extent of red fluorescence (ΔR) in caries lesions to ICDAS II scores.

Methods: Ethical approval was obtained. 83 subjects attending an oral surgery clinic for extraction of posterior teeth were recruited yielding 350 unrestored human posterior teeth for the study. The teeth were scored using ICDAS II; images of 3 surfaces (occlusal, buccal & lingual) for each tooth using different camera systems (White-light digital camera and QLF) and periapical radiographs were obtained. Following extraction, teeth were sectioned and subjected to direct histological examination.

Results: QLF was able to significantly differentiate all ICDAS II severity scores ($p < 0.001$). ICDAS II correlated best with ΔF (%) (0.843 on occlusal, 0.846 on buccal and 0.907 on lingual surfaces) followed by ΔQ (%.mm^2) (0.800 on occlusal. 0.870 on buccal and 0.897 on lingual surfaces). ΔR (%) correlated less well for early lesions and correlated better for more advanced lesions.

Conclusion: QLF proved most useful for classifying lesions using ΔF and may aid clinical decision making. Red fluorescence correlates well with more advanced lesions and could be useful as a secondary factor in caries analysis